UNIVERSITY OF MICHIGAN
FEB 13 1953
MEDICAL LIBRARY

Vol. 42, Nos. 3, 4 January 1953

PARASITOLOGY

(Founded by G. H. F. Nuttall)

EDITED BY

D. KEILIN and P. TATE

IN CONJUNCTION WITH

A. BISHOP
F. J. BROWN
P. A. BUXTON
E. HINDLE
C. A. HOARE
G. LAPAGE
K. M. SMITH

CAMBRIDGE UNIVERSITY PRESS
LONDON: BENTLEY HOUSE, N.W.1
NEW YORK: 32 EAST 57TH STREET, 22

Thirty-seven Shillings net

Issued 8 *January* 1953

A NEW NATURALIST SPECIAL VOLUME

Fleas Flukes & Cuckoos

A Study of Bird Parasites

Miriam Rothschild and Theresa Clay

"**All naturalists must read this book,** and they will enjoy not only the fascinating and immense body of information that it contains, but also the delightful style in which it is written."
—THE TIMES LITERARY SUPPLEMENT

"**Surely the best and most complete** text-book on the three groups of parasite which provide the title. It is of extraordinary interest and written by two most distinguished zoologists who have specialised in the subject." —SHOOTING TIMES

With 127 illustrations. *21s.*

This is the 3rd reprint in 7 months!

COLLINS

MICHROME

MICROSCOPIC STAINS

Organic Reagents etc.

Azocarmine	Janus Green, B
Brilliant Cresyl Blue	Lacmoid
Cedarwood Oil	Leishman Stain
Field's Stain	Nile Blue
Giemsa Stain	Rosolic Acid
Haematoxylin	Trypan Red, etc.

Water analysis tablets

Stains and Reagents for Fluoresence Microscopy

EDWARD GURR, LTD.

42 Upper Richmond Road,
East Sheen, London, S.W. 14

Telephone: Prospect 7606 & 8051
Cables: Micromlabs London

SERVICE, UNIFORM HIGH QUALITY, RELIABILITY, IMMEDIATE DELIVERY

Now ready: **Microscopic Staining Techniques**
Part 1 (24 pp.) 1s. 6d. Part 2 (62 pp.) 3s. 6d.
Part 3 (64 pp.) 3s. 6d.

The TREMATODA

BEN DAWES

A detailed study of the trematode parasites of vertebrate animals such as the advanced student of zoology dissects in the laboratory, intended to give zoologists and parasitologists a more useful account of the Trematoda than can be found in existing works. The author is Lecturer in Zoology at King's College, London.

75s. net

CAMBRIDGE UNIVERSITY PRESS

BY I

I. Intr
II. The
licej
III. The
to t
IV. The

The follo
of studie
British t
taken wi
some ear
Linnaeus
meagre,
pects wer
I. canisu
1895, *I.*
ovatus N
I. frontal
Ceratixod
vespertili

The st
Ixodes he
where (A
for comp
ticularly
has been
structure
and host
Warburto
aspect in
tion. Sno
literature
apart fro
1946) of t
tion on th

Most ac
able ovip
the egg l
moments

VOLUME 42, NOS. 3, 4 JANUARY 1953

THE MORPHOLOGY OF THE BRITISH PROSTRIATA WITH PARTICULAR REFERENCE TO *IXODES HEXAGONUS* LEACH. II

BY D. R. ARTHUR, *The Department of Zoology, King's College, University of London*

(With 40 Figures in the Text)

CONTENTS

	PAGE
I. Introduction	161
II. The palps and hypostome in *Ixodes trianguliceps* and *I. canisuga*	162
III. The capitulum of the Prostriata in relation to their hosts	163
IV. The scutum	168
V. Gené's organ	176
VI. Mechanism of stretching during feeding	180
VII. Alloscutal orifices and appendages	182
VIII. Summary	184
References	185

I. INTRODUCTION

The following account presents the results of a series of studies on the morphology and ecology of some British ticks (Prostriata) which have been undertaken with the object of amplifying and correcting some earlier versions. Apart from *Ixodes ricinus* Linnaeus, 1758, our knowledge of British ticks is meagre, and accordingly certain morphological aspects were investigated in *I. hexagonus* Leach, 1815, *I. canisuga* Johnston, 1849, *I. trianguliceps* Birula, 1895, *I. melicola* Schulze & Schlottke, 1929, *I. unicavatus* Neumann, 1908, *I. caledonicus* Nuttall, 1910, *I. frontalis* Panzer, 1795, *I. passericola* Schulze, 1933, *Ceratixodes uriae* White, 1852, and *Eschatocephalus vespertilionis* Neumann, 1901.

The structure of the capitulum of all stages of *Ixodes hexagonus* is considered in some detail elsewhere (Arthur, 1951*a*) as it provided a suitable type for comparison with other British species, particularly with regard to the capitulum. Emphasis has been placed on the structural adaptations of this structure in the various ticks in relation to feeding and host selection. As long ago as 1911, Nuttall, Warburton, Cooper & Robinson remarked that this aspect in the Ixodidae required further investigation. Snodgrass (1948) has given a summary of the literature concerning the feeding organs of ticks but apart from reference to the writer's account (Arthur, 1946) of the trophi of *I. ricinus*, no further information on the Prostriata is available.

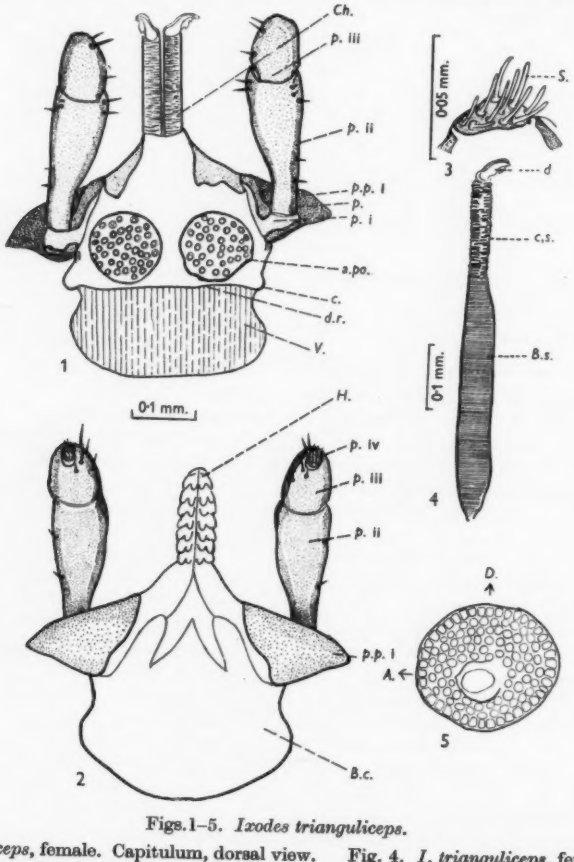
Most accounts of ticks refer briefly to the remarkable oviposition movements of the females. After the egg leaves the vagina it is received for a few moments by the glandular organ of Gené, which is everted shortly before egg-laying. This structure was first observed just over a century ago, but our knowledge of its morphology and its method of eversion still remains meagre. Most of the work has been descriptive, but recently Lees & Beament (1948) have made a study of this structure in the Argasid tick, *Ornithodoros*. My observations have been made on *I. hexagonus* as a representative of the Ixodidae, and they are more detailed than anything hitherto attempted for this species.

Many measurements have to be made in a morphological study of this kind. These proved useful, as the degree of variability at any stage of tick development could then be determined, and in addition the mean growth rate from stage to stage together with changes in form could be ascertained. This was applied in particular to the scuta and to the segments of the legs.

In the preparation of this work I am particularly indebted to Dr Ben Dawes for his guidance and for his unlimited patience in supervising my studies. Dr P. Tate very kindly read the typescript and his criticisms are much appreciated. In addition to my own material, I received supplies of British ticks and hosts from Mr G. B. Thompson; the British Museum (Natural History) through the courtesy of Mr E. Browning; Mr W. Thomas, Ammanford; Mr M. Froud, M.Sc., Wye; Mr J. Mugeridge, Isle of Grain; Mr G. Saunders, Gavestone; Mr J. Ash, Silwood Park and Mr Colin Matheson, M.A., M.Sc., Cardiff. Dr Glen M. Kohls, Rocky Mountain Laboratory, Montana, U.S.A., lent me specimens of American ticks for comparison with those of Britain. To all these people I am deeply indebted for their courtesy and assistance at all times.

II. THE PALPS AND HYPOSTOME IN *IXODES TRIANGULICEPS* AND *IXODES CANISUGA*

Nuttall *et al.* (1911) wrote 'the first article of *I. tenuirostris* (*I. trianguliceps*) (is) in the form of a stout spur, directed transversely outward and fused with the basis'. I find that the first palpal article of the female of *I. trianguliceps* is a distinct segment with a suture between it and the basis capituli (Fig. 1). The subterminal segment is shorter, being 0·123 mm. (0·104–0·142 mm.) long with its greatest breadth 0·098 mm. (0·089–0·104 mm.) at the base, so that the second segment is about 1·6 times as long as the third, and the breadth of the third article slightly less than that of the second. The mesial concavity of the palp is deeper on the subterminal article than on the second. The proximal palpal segments in the nymph are likewise large, having their bases arranged



Figs. 1–5. *Ixodes trianguliceps.*

Fig. 1. *I. trianguliceps*, female. Capitulum, dorsal view.
Fig. 2. *I. trianguliceps*, female. Capitulum, ventral view.
Fig. 3. *I. trianguliceps*, female. Apex of palpal article I.
Fig. 4. *I. trianguliceps*, female. Chelicera.
Fig. 5. *I. trianguliceps*, female. Spiracle.

It is large, with the apex directed laterally and the breadth/length ratio is 3·3/1·4. From the base and underneath the first article there is a laterally projecting plate and this, presumably, is the spur to which earlier writers alluded. A similar, but smaller, plate is present in *I. canisuga* (Fig. 14). In the female of *I. trianguliceps* the second article is 0·19 mm. (0·18–0·20 mm.) long, with a maximum breadth of 0·103 mm. (0·095–0·108 mm.) apically. obliquely to the long axis of the basis capituli (Fig. 6). The ratio of the length of the third article to that of the second is as 1·0/1·3, while the basal breadth of the third is slightly less than the distal end of the second. The first article in the larva (Fig. 9) is similar to that of the nymph, but the basiventral plate points laterally, is triangular in outline and relatively smaller than in the nymph. There is no evidence of a suture between the second and third

palpal articles, and their combined length is nearly three times the maximum breadth.

In *I. ricinus*, *I. hexagonus* and *I. canisuga* the fourth article is a small cap of cuticle, which bears a number of sensilla of the 'chemo-receptor type' (Lees, 1948). The fourth palpal article of *I. trianguliceps* has rigid walls; its mesial surface is inclined at an angle to the longitudinal axis of the palp and is longer than the lateral surface. The apex of the terminal article is paler than the walls and bears sensillae (Fig. 3). These are often slightly curved, have rounded tips, thin delicate walls and are of about equal length *inter se*. This article is intucked into the subterminal article and can be protruded and retracted. In all the material of *Ceratixodes uriae* which I have examined, the fourth article is permanently extended. Hence *Ixodes trianguliceps* seems to represent an intermediate condition between *Ceratixodes uriae* and *Ixodes hexagonus*-like types.

The female of *I. canisuga* has a broad hypostome, the length/breadth ratio being 2·0/1·0. It is more pointed than in *I. hexagonus* and marginally has seventeen to nineteen conspicuous teeth which extend to the apex. From the proximal to the distal end they are arranged in five rows of 2/2 files separated by a median, unarmed area, one row of 3/3 files, one row of 4/4 files, after which the ventral teeth become minute. The male hypostome bears seven or eight rows of crenulations which stretch across its ventral surface. Distally it is bifid with three pointed, forwardly directed teeth on the outer edge of each ramus. The basal teeth of the hypostome originate on a level with the base of the first palpal article. The significance of this will be discussed later. The hypostome of the female of *I. trianguliceps* is spatulate, has curved profile lines and terminates in a rounded apex; the length/breadth ratio is 2/1. The figure of Neumann (1899), repeated by Nuttall *et al.* (1911), depicts this as tapering to a point, and similar to the hypostome of *Eschatocephalus vespertilionis*. This is incorrect. The teeth are rounded and occupy the entire ventral surface where they are arranged in eight rows of 2/2 files (Fig. 2). These teeth decrease in size distally and there is no corona. Larvae and nymphs usually have rounded or truncated hypostomes which are of little value in penetrating the host skin, but, when inserted, the backwardly projecting teeth secure the tick in position.

III. THE CAPITULUM OF THE PROSTRIATA IN RELATION TO THEIR HOSTS

(*a*) *Rostrum*

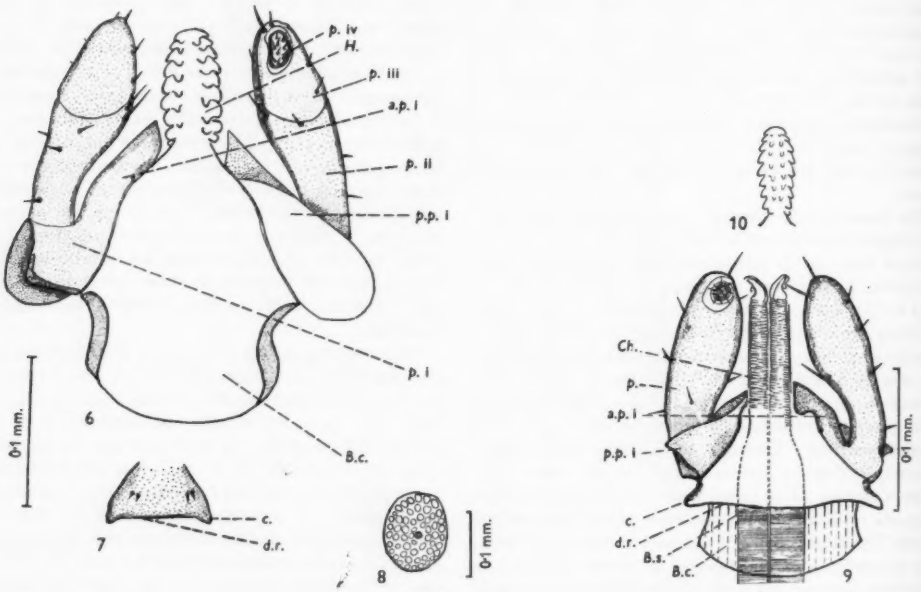
The front margin or rostrum of the basis capituli in the British Prostriata varies in shape (Figs. 15, 16) and affords a basis for a broad classification. The first group includes *Ceratixodes uriae*, *Ixodes uni-cavatus*, *I. caledonicus* and *I. canisuga*. The rostrum does not usually reach to the tip of the first palpal segment, thus the hypostome and chelicerae seem to originate at or near the base of the palp. In these ticks the teeth on the hypostome extend back to the front margin of the tectum. Hence maximum penetration of the host-tissue cannot exceed the length of the hypostome. The first palpal articles lie antero-dorsally. Each article is a complete ring visible from all aspects and in all stages. The capitulum of this group resembles that of the Notostigmata, as described by With (1904), and while in no sense primitive, unquestionably shows an early stage in the evolution of the acarine capitulum. The rostrum in the Notostigmata is a small fold of the dorsal integument overlying the bases of the pedipalpal coxae to form the basis capituli. The trochanters have no spurs or projections. The rostrum of *I. canisuga* is of interest because it is produced forward to the level of the apex of the pedipalpal trochanter. Apart from this species, all the ticks in this group are parasitic on birds. *I. canisuga* occurs on sand martins, bank swallows, foxes and dogs, and the available information shows that copulation does occur in nests of sand martins, and in dog kennels in northern England.

Allied to this group are species with forward projections from the pedipalpal trochanters, which are free from the basis capituli along their mesial surfaces. We have no British representatives of this group, and the structure is exemplified by all stages of the American species, *I. auritulus*, and the females of the Australian species *I. tasmani*. The articulation of the palp in *I. auritulus* is between the first and second segments, and the basal segment is prolonged forwards on its mesial surface and is parallel with the side of the hypostome for nearly half the length. Consequently, the effective attachment surface is slightly more than one-half the length of the hypostome. The rostrum in *I.* (= *Endopalpiger*) *tasmani victoriae* Sch. resembles that of *Ceratixodes uriae* with similar palpal segments to those of *Ixodes auritulus*. The hypostome is spatulate and broadest just behind the tip. Its teeth are small proximally and, except for three rows of subapical teeth, increase in size towards the tip. Here again it would seem that the larger hooked teeth are the main fixation structures. The lobe-like projections in this species embrace the mouthparts. Schulze considered these to be the pedipalpal trochanters, and he interpreted the long third segment of each palp as the combined second and third segments. If trochanter spurs are present in mature ticks, they also occur in the immature stages, but no known species has spurs in the adult and is without them in the immature stages. The converse is true, however, for a number of species.

The third group of ticks has the trochanter spur

partially or completely fused with the basis. In the immature stages fusion is partial, but in adults it is complete as in *I. trianguliceps* and *I. angustus*. The anterior spurs of the palpal trochanter in the larvae and nymphs of *I. trianguliceps* are partially fused with the basis, but are free distally and form short extensions on either side of the chelicerae. The relative size of the first segment in the female is smaller than in the immature stages, and the anterior projection is here separated from the tro-

moves beyond the intercoxal bridge, the cheliceral digit can only function if there is an increase in the relative length of the chelicera' shaft. This is borne out by the data in Table 1, where the shaft length is expressed as a ratio of the distance between the bases of the palps. With the lengthening of the shaft there is also a suggestion that the relative width decreases as seen by comparing *I. trianguliceps* with *Ceratixodes uriae*, and *Eschatocephalus vespertilionis* with *Ixodes canisuga*.



Figs. 6–10.

Fig. 6. *I. trianguliceps*, nymph. Capitulum. The left palp is drawn from above and the right from below.
Fig. 7. *I. trianguliceps*, nymph. Capitulum, posterior portion. Fig. 8. *I. trianguliceps*, nymph. Spiracle.
Fig. 9. *I. trianguliceps*, larva. Palp on left-hand side seen from below, the right palp from above.
Fig. 10. *I. trianguliceps*, larva. Hypostome.

chanter, becoming incorporated into the basis as the shoulder, or 'sella'. Both *I. trianguliceps* and *I. angustus* are parasitic on small mammals of the mouse group.

The tectum in the fourth group is produced beyond the distal end of the palpal trochanter, and the hypostome and chelicerae appear to originate in front of the apex of this segment. The length of the rostrum in this group varies as shown in Figs. 15 and 16 and they may be confined to one mammalian host or have a wide host range. As the rostrum becomes longer the palpal base and the intercoxal bridge do not move forward correspondingly. Since the front margin of the bulbous base of the chelicera never

(*b*) *The digit*

The structure of the digit is of little importance in systematic work (Nuttall *et al.* 1911), though it may be of considerable value in host selection. The lengths of the digits are given in Table 1 and the number of teeth on the female digits in Table 2. Analysis of these data indicates that the species having the longest and most toothed digits have the widest host range, e.g. *I. ricinus* and *I. hexagonus*, whilst those with shorter digits and fewer teeth are limited to one group of hosts, e.g. *I. caledonicus* and *I. trianguliceps*. Occupying an intermediate position are species which have long digits and a small number of teeth or vice versa (Fig. 16).

Table 1. *Measurements (in mm.) of the cheliceral shafts and digits of some British Prostriata in relation to the rostrum*

Species	Stage	Length of cheliceral shaft (*S*)	Distance between outer margins of palpal article I (*P*)	*S*/*P*	Length of digit (mm.)
C. uriae	F	0·37	0·43	0·86	0·123
C. uriae	N	0·24	0·29	0·83	0·080
C. uriae	L	0·13	0·19	0·68	0·040
I. caledonicus	F	0·32	0·46	0·69	0·089
I. caledonicus	N	0·18	0·26	0·69	0·064
I. caledonicus	L	0·11	0·18	0·62	0·035
I. canisuga	F	0·34	0·39	0·87	0·094
I. canisuga	N	0·17	0·31	0·55	0·064
I. canisuga	L	0·09	0·15	0·60	0·021
I. hexagonus	F	0·498	0·496	1·00	0·163
I. hexagonus	N	0·26	0·32	0·81	0·073
I. hexagonus	L	0·128	0·171	0·75	0·045
I. ricinus	F	0·55	0·43	1·27	0·166
I. ricinus	N	0·25	0·25	1·00	0·083
E. vespertilionis	F	0·43	0·43	1·00	0·131
E. vespertilionis	N	0·25	0·26	0·96	0·067
E. vespertilionis	L	0·21	0·18	1·16	0·038
*I. trianguliceps**	F	0·22	0·24	0·91	0·051
I. trianguliceps	N	0·12	0·21	0·59	0·032
I. trianguliceps	L	0·079	0·144	0·55	0·019

* The relationship of the palps to the capitulum is discussed in § III.

Table 2. *Number of teeth on the digit of females of some British Prostriata*

Species	No. of teeth on digital articles: Internal article	Dorsal process of internal article	External article
I. caledonicus	1	2	4
C. uriae	1	2	4
I. canisuga	1	2	5
I. hexagonus	2	2	5
I. trianguliceps	1	2	3
I. ricinus	2	2	8
E. vespertilionis	2	1–1 (?)	7

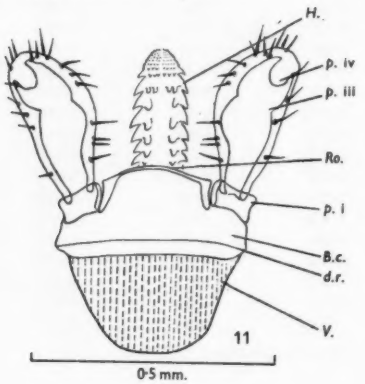
(c) *The capitulum in relation to tick distribution on the host*

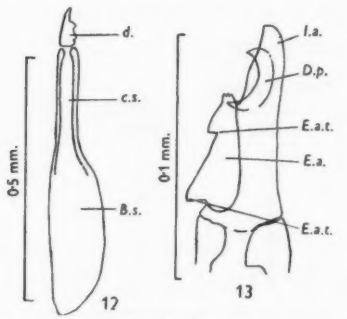
Female ticks (*I. ricinus*) on sheep are generally attached to the head, legs, the bare and short woolled margins of the axillary and inguinal areas, but they do not attach in the long wool of the withers, back, rump and flanks. The nymphs occupy the distal parts of the ears, snout, chin and lower arm, whilst the larvae feed at the tips of the ears, round the nostrils, lips and fetlock (Milne, 1947).

Extensive observations have shown that the backs and flanks of cattle are usually free of ticks (*I. ricinus*), and that feeding female ticks are concentrated in the axillae of the forelegs and particularly in the groin. The principal region of infestation by *I. hexagonus* on hedgehogs is around the anus and genital organs. This was so in 85% of the infested animals examined, and of these 9% also had ticks on the ears and jaws. Some 15% had ticks on these sites only. A similar distribution was observed on weasels and stoats, where 80% of the ticks were found in the perineum and 18% on the ears and face.

	Ears	Face	Axillae (fore)	Axillae (hind)	Anus	Genital region
Hedgehogs	8	7	—	3	25	57
Weasels	7 } 15	1 } 3	2 } 2	5 } 7	13 } 33	21 } 40
Stoats	8 }	2 }	— }	2 }	20 }	19 }

A similar picture is presented by other acari, as shown by Elton & Keay (1936) and Jones (1950) for harvest mites, by Hughes (1949) and Sudd (private communication) for rat mites, and by Audy (private communication) for certain scrub-typhus mites. All these species, like *I. hexagonus*, penetrate into the dermis for their food supply. The tick, *Dermacentor reticulatus*, however, occurs on the backs and flanks of cattle, but as Talice (1930) and Gregson (1937) showed, the hypostome in this species rarely penetrates deeply beneath the epidermis.





Figs. 11–13.

Fig. 11. *I. canisuga*, female. Capitulum from dorsal side. Hypostome seen in transparency.
Fig. 12. *I. canisuga*, female. Chelicera.
Fig. 13. *I. canisuga*, female. Digit.

Various theories have been put forward to account for the distribution of acari on their hosts. Jones suggested that the factors influencing the site of attachment of *Trombicula autumnalis* on man are the thinness of the skin, warmth and humidity, but observations show that ticks become attached on exposed sites, e.g. the ear. Experimental evidence likewise does not confirm Jones's suggestion, for when attached to the host the tick gains water from ingested blood and loses it in the excrement (Lees, 1946).

Milne (1947) observed a zonation of ticks on the host, and the larger the tick stage the farther does its attachment site recede from the arrival point. This recession, he suggested, 'may be correlated with gradients in friction. Thus on proceeding from lips back to cheeks, we find external friction decreasing and hair length (cover) increasing. Lessened external friction without increase in cover may suffice.' Lees (1948) expressed the opinion that females required a more prolonged stimulation for attachment than do nymphs, and nymphs than larvae. The writer believes that these three theories may contribute something to the truth, and in addition that the micro-structure of the host skin deserves further investigation. The micro-structure of the skin depends on the part of the body from which the sample

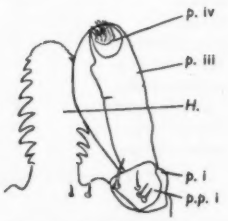


Fig. 14. *I. canisuga*, female. Palp seen from below to show ventral plate.

is taken (Chiodi, 1933; Braun, Reismann, Ivanov & Rjabinen, 1932; Carstens & Kinzelbach, 1933; Nemec, 1940; Bernshtein, 1948). The skin of cattle is thickest along the back (4·4–5·2 mm.) and has crossing fibre bundles and is therefore firm. Along the rump and flanks the thickness varies from 3·9 to 5·2 mm., and the fibre bundles cross less frequently. In the axillae the skin is thinnest, ranging from 1·8 to 2·4 mm. with the fibres arranged horizontally. Sections of skin from different parts of the body of a stoat agree with this, and the thinnest skin is found in the ear, axilla and groin. Horizontal fibres predominate in these regions and the tissue is open and lacks firmness. The digits of each tick stage vary in size (Table 1), but their dentition is similar, and accordingly the larger the digit the more effective will be its penetrating power. Larvae and nymphs of *Ixodes ricinus* occur on the lips, jaws and tips of the ear in cattle, and larvae and nymphs of *I. hexagonus* occupy similar sites on small mammals. The axillae and groins of these hosts are usually selected by the females of both species. When small mammals, e.g. the rabbit, are parasitized by *I. ricinus*, the tick is frequently found on the back and occupies this position, presumably, because the skin on the rabbit's back is thinner than the correspond-

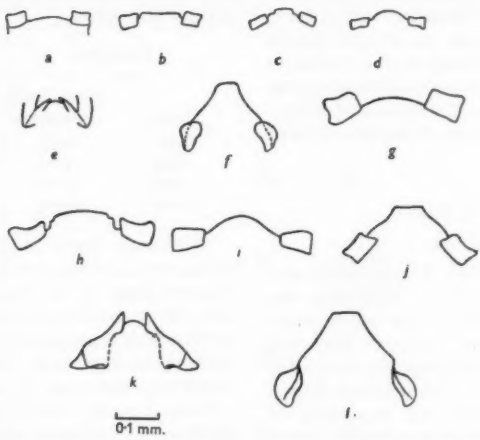


Fig. 15. The rostrum of some immature tick stages. (*a*)–(*f*) larvae: (*a*) *C. uriae*; (*b*) *I. caledonicus*; (*c*) *I. canisuga*; (*d*) *I. hexagonus*; (*e*) *I. trianguliceps*; (*f*) *E. vespertilionis*. (*g*)–(*l*) nymphs: (*g*) *C. uriae*; (*h*) *I. canisuga*; (*i*) *I. hexagonus*; (*j*) *I. ricinus*; (*k*) *I. trianguliceps*; (*l*) *E. vespertilionis*.

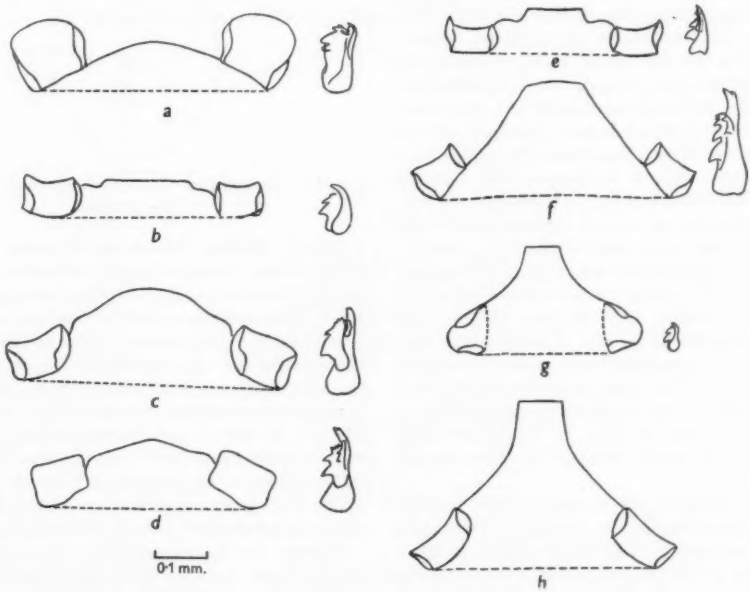


Fig. 16. The rostrum of female ticks: (*a*) *C. uriae*; (*b*) *I. caledonicus*; (*c*) *I. hexagonus*; (*d*) *I. melicola*; (*e*) *I. canisuga*; (*f*) *I. ricinus*; (*g*) *I. trianguliceps*; (*h*) *E. vespertilionis*.

ing area of either cow or sheep. Since all stages of ticks are liable to arrive on any part of the host, e.g. ticks may be picked up when the cow is lying down in tick-infested pastures, we should expect ticks to be randomly distributed and not concentrated in particular parts. It would therefore appear that the size of the digit, and thickness and microstructure of the skin, may determine in part, at least, the choice of feeding sites.

IV. THE SCUTUM

(*a*) *Size and form*

The records of Neumann (1899), Wheler (1899) and Nuttall *et al.* (1911) showed that intraspecific size variation occurs in the Prostriata, but the methods of measuring used by different authors were not comparable. In the present work the scuta are used to study the size and variability of the different stages. Moreover, the results discredit the suggestion of Nuttall *et al.* (1911) that the larvae of *Ixodes ricinus* and *I. hexagonus* 'have the same essential characters of the nymph, and the nymph those of the adult'.

The sizes of the scuta of the males and females of *I. hexagonus* and *I. ricinus* have been given by Nuttall *et al.* (1911) but whether they were measured *in situ* or after removal is not stated. Stretching of the body during feeding may reduce the curvature and alters the orientation of the scutum. Hence, measurements of the scutum of unfed, partially fed and fully fed ticks *in situ* may give results which are not comparable. In the present work the scuta were separated from the dorsum and mounted flat on slides under cover-slips. Sometimes this separation was difficult, particularly in larvae, but staining with 1 % alcoholic eosin or 1 % acid fuchsin proved useful in differentiation, as the alloscutum stained more intensely and more rapidly than the scutum. The length (L) of the scutum was measured from the level of the scapular apices to the posterior edge, and at its greatest breadth (B_2); B_1 was the distance between the scapulae (Fig. 17). A micrometer eyepiece was used for measuring, and calibrated against a micrometer scale. The mean scutal increase from stage to stage was also ascertained in this way. Many specimens of *I. ricinus* and *I. hexagonus* were examined but only small batches of other species were available.

The results obtained for *I. ricinus* (see Arthur, 1949) and *I. hexagonus* in the present investigation are given as correlation diagrams in Figs. 18–20.

The breadth of the scutum usually exceeds the length in the larvae of *I. ricinus*, *I. hexagonus*, *I. canisuga*, *I. caledonicus* and *I. trianguliceps*, and thus the ratio L/B_2 is almost always less than unity. The length of the larval scutum of *Ceratixodes uriae* and *Eschatocephalus vespertilionis* is greater than the breadth, and likewise the length of the nymphal scutum in most British species. In *I. ricinus* and *I. hexagonus* the length-breadth ratio of the nymphal scutum is as follows:

I. ricinus		*I. hexagonus*	
L/B_2	Percentage occurrence	L/B_2	Percentage occurrence
0·97	2·62	0·97	1·59
0·98	4·70	0·98	4·77
0·99	3·71	>1·00	93·74
>1·00	88·97	—	—

The larger scuta of these two species have a truncated appearance which contrasts with the more rounded form of the 'dwarf'. A comparison of the data for *I. ricinus* and Figs. 18–20 shows a greater vertical and horizontal scatter in nymphs than in larvae, and in females than in nymphs, so that the variability increases as maturity is attained.

Nuttall *et al.* (1911) stated that the scuta of females of *I. ricinus* measured 1·3 × 1·15 mm. or 1·4 × 1·05 mm. Plots of the length/breadth ratios should therefore show peaks at 1·13 and 1·33. I examined 898 specimens of *I. ricinus*, of which 470 were

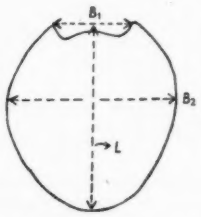


Fig. 17. Diagram to show methods used in measuring the scutum.

collected during September–October and 428 in March–June. Scuta of ticks collected from sheep, cattle, horses and dogs, were also examined. Neither set of data confirms the earlier results, but rather suggests that the distribution of size of the scuta about the mean is not regulated by any special factor and the statistical material is homogeneous. Similar results are deduced from the data on *I. hexagonus* (Fig. 21). In all British species the length of the female scutum is always greater than the breadth. From the mean values of the length and breadth of scuta in Table 3 the percentage increase in size at successive stages is determined and the results given in Table 4.

Except in *Eschatocephalus vespertilionis*, these results show that the percentage increase in mean length of the scutum from larva to nymph is greater than the mean breadth (B_2) and that this increase is not constant in all species. Nevertheless, it is adequate, except possibly in *Ixodes canisuga*, to alter the length/breadth (L/B_2) ratio from less than unity in the larvae to a value either equal to, or greater

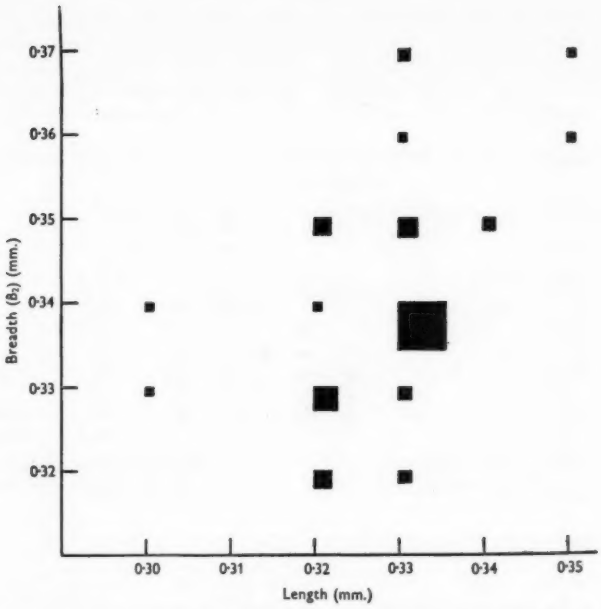


Fig. 18. *I. hexagonus.* Correlation diagram of larval scuta. The area of each square is proportional to the number of samples occurring at each point.

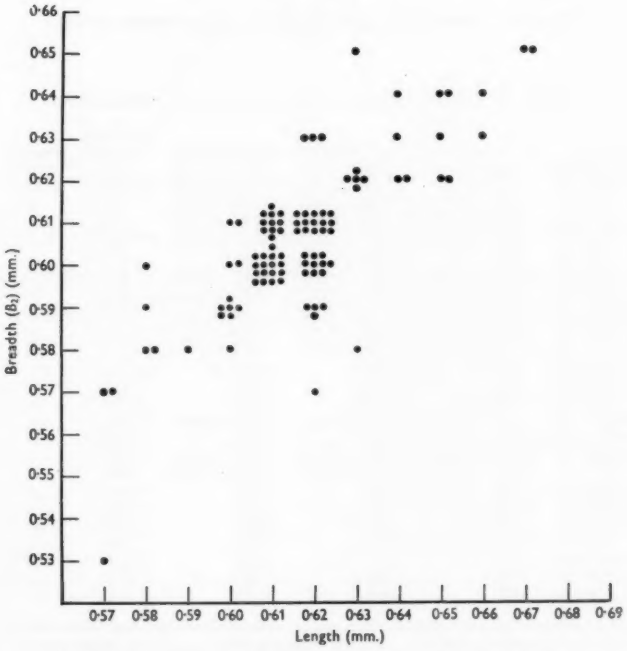


Fig. 19. *I. hexagonus.* Correlation diagram of nymphal scuta.

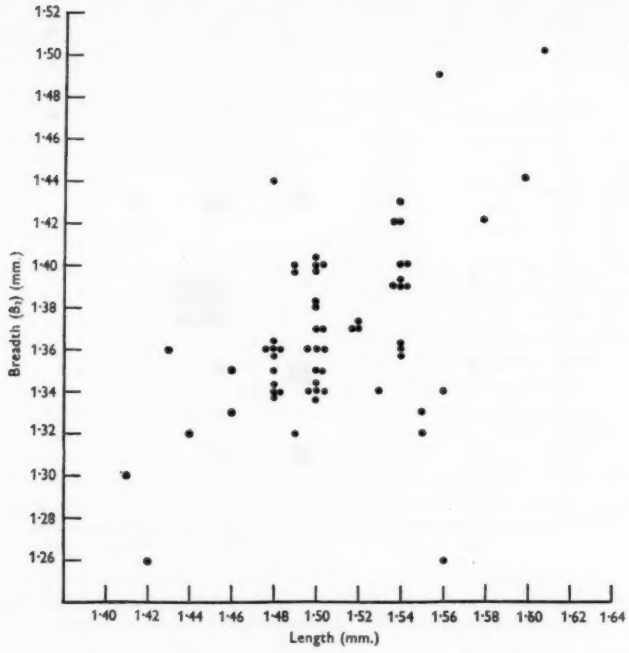


Fig. 20. *I. hexagonus.* **Correlation diagram of female scuta.**

Table 3. *The characters of the scuta of some British ticks*

Species	Stage	s.s./s.a.	Length (L)	Breadth (B_2)	Breadth (B_1)	Greatest breadth	Scapulae	Cervical grooves	Lateral carinae	No. of scuta examined
I. hexagonus	F	8–11/13–14	1·508 ±0·04	1·368 ±0·043	0·69 ±0·036	W.F.	*p*	*pr*	+	See figs. 18–20
I. hexagonus	M	8–11/13–14	3·08 ±0·02	1·63 ±0·024	0·43 ±0·020	W.M.	*r*	*pr*	−	
I. hexagonus	N	7– 8/ 8–10	0·62 ±0·019	0·606 ±0·018	0·316 ±0·016	W.F.	*r*	*pr*	−	
I. hexagonus	L	6– 8/ 8–10	0·327 ±0·008	0·34 ±0·012	0·17 ±0·008	W.F.	*r*	*pr*	−	
C. uriae	F	14–17/60–70	1·36 ±0·02	1·0 ±0·015	0·57 ±0·017	W.F.	*r*	*pr*	−	20
C. uriae	M	7–10/25–30	3·87 .	2·26 .	0·55 .	W.F.	*r*	*pr*	−	4
C. uriae	N	7– 8/20–24	0·73 ±0·005	0·5 ±0·0045	0·32 ±0·005	W.F.	*r*	*pr*	−	20
C. uriae	L	4– 7/11–16	0·40 ±0·0012	0·28 ±0·0013	0·18 ±0·0016	W.F.	*r*	*pr*	−	40
I. trianguliceps	F	10–15/17–20	0·824 ±0·0102	0·768 ±0·020	0·352 ±0·014	W.B.	*p*	*ab*	+	15
I. trianguliceps	N	8– 9/13–17	0·464 ±0·005	0·408 ±0·009	0·144 ±0·02	W.B.	*p*	*ab*	+	20
I. trianguliceps	L	10–11/12–15	0·256 ±0·0022	0·288 ±0·003	0·088 ±0·0029	W.B.	*r*	*ab*	+	25
I. caledonicus	F	17–19/17–20	1·168 .	0·984 .	0·464 .	W.F.	*r*	*pr*	−	2
I. caledonicus	N	9–10/10–12	0·56 .	0·56 .	0·144 .	W.F.	*r*	*pr*	−	5
I. caledonicus	L	5– 7/ 8–10	0·32 .	0·376 .	0·096 .	W.F.	*r*	*pr*	−	3
I. canisuga	F	15–17/15–18	1·09 ±0·019	1·01 ±0·059	0·60 ±0·063	W.F.	*r*	*pr*	+	18
I. canisuga	M	7– 9/ 7– 9	2·47 .	1·44 .	0·37 .	W.M.	*r*	*ab*	−	2
I. canisuga	N	8–10/12–15	0·58 ±0·0098	0·59 ±0·0098	0·32 ±0·007	W.F.	*r*	*pr*	+	18
I. canisuga	L	6– 8/ 7– 9	0·32 ±0·0018	0·34 ±0·003	0·16 ±0·0051	W.F.	*r*	*pr*	−	78
I. ricinus	F	37–39/40–44	1·38 .	1·25 .	0·57 .	W.M.	*p*	*pr*	−	See figs. in Arthur (1960)
I. ricinus	M	30–40/35–40	1·78 .	1·15 .	0·54 .	W.B.	*r*	*ab*	−	
I. ricinus	N	11–13/17–26	0·62 .	0·59 .	0·24 .	W.M.	*r*	*pr*	−	
I. ricinus	L	7– 9/15–19	0·33 .	0·38 .	0·14 .	W.M.	*r*	*pr*	−	
E. vespertilionis	F	10–60/70–95	1·56 .	1·0 .	0·63 .	W.F.	*r*	*pr*	−	2
E. vespertilionis	N	9–12/20–26	0·89 .	0·56 .	0·20 .	W.F.	*r*	*pr*	−	4
E. vespertilionis	L	7– 9/16–18	0·45 .	0·32 .	0·10 .	W.M.	*p*	*pr*	−	5

s.s/s.a., ratio of length of scutal bristles to alloscutal bristles; W.F., widest in front of middle; W.M., widest about middle; W.B., widest behind the middle; *pr*, present; *ab*, absent; *p*, pointed; *r*, rounded; +, lateral carinae present; −, lateral carinae absent.

than, unity in the nymphs. In other words, after moulting, the scutum alters in shape (Figs. 22–24). Changes are also apparent in the form of the scutum after the nymphs moult, and these, likewise, are not constant in all the British Prostriata. This is seen

The female scutum (Fig. 24*l*) is about 1·1 times as long as broad, with its greatest breadth at about two-thirds the distance from the scapulae, and in front of this the antero-lateral borders are slightly concave. The hind-margin is broadly convex. The

Table 4. *Percentage increase in the length and breadth of the scutum of successive stages of some British species*

Species	Percentage length increase			Percentage breadth (B_1) increase			Percentage breadth (B_2) increase		
	L–N*	N–F†	N–M‡	L–N*	N–F‡	N–M‡	L–N*	N–F†	N–M‡
I. ricinus	89	123	187	71	138	125	59	112	95
I. hexagonus	89	143	.	.	.	.	80	126	.
C. uriae	83	86	430	78	78	72	82	96	324
I. caledonicus	75	109	§	50	222	§	49	76	§
I. canisuga	81	88	327	100	90	16	73·5	71	144
I. trianguliceps	81	78	§	64	144	§	42	88	§
E. vespertilionis	50	75	§	100	.	§	75	79	§

* Increase from larva to nymph. † Increase from nymph to female.
‡ Increase from nymph to male. § No males available.

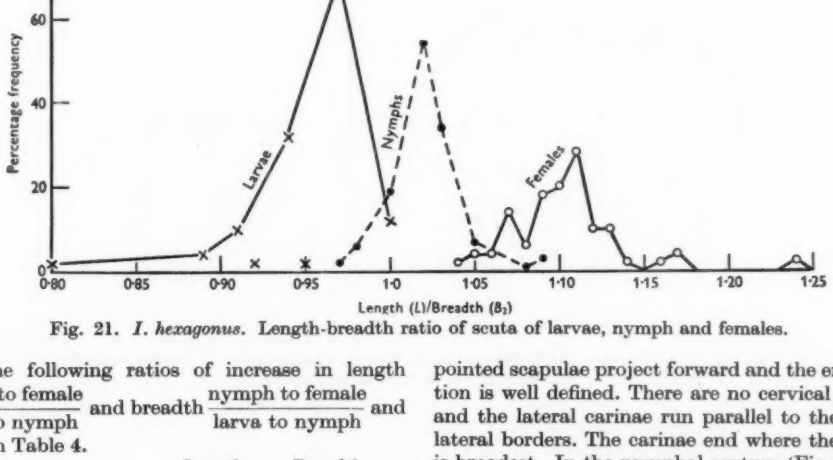


Fig. 21. *I. hexagonus*. Length-breadth ratio of scuta of larvae, nymph and females.

from the following ratios of increase in length $\frac{\text{nymph to female}}{\text{larva to nymph}}$ and breadth $\frac{\text{nymph to female}}{\text{larva to nymph}}$ and based on Table 4.

	Length $\frac{\text{N–F}}{\text{L–N}}$	Breadth $\frac{\text{N–F}}{\text{L–N}}$
I. ricinus	1·4	2·0
I. hexagonus	1·6	1·56
C. uriae	1·04	1·17
I. caledonicus	1·45	1·55
I. canisuga	1·09	0·96
I. trianguliceps	0·96	2·9
E. vespertilionis	1·50	1·05

The material of *I. trianguliceps*, although inadequate to justify statistical treatment, is sufficient to amplify and correct the descriptions of Neumann (1899) and Nuttall *et al* (1911).

pointed scapulae project forward and the emargination is well defined. There are no cervical grooves, and the lateral carinae run parallel to the antero-lateral borders. The carinae end where the scutum is broadest. In the nymphal scutum (Fig. 24*e*) the greatest breadth is more than one-half of the length from the scapulae, with the antero-lateral margins straight and converging. The posterior margin is more rounded than in scuta of females, but the emargination and scapulae are similar. The length of the larval scutum is less than the breadth; the length/breadth ratio is 0·90/1·0 (Fig. 24*a*). The scutum is ovate and the greatest breadth is slightly behind the middle. The antero-lateral borders are slightly convex, the emargination distinct but not so well defined as in nymph or female. The scapulae are broad protuberances. The lateral carinae extend from the scapulae to the widest points. There are no cervical grooves.

The scuta of *Ceratixodes uriae* and *Eschatocephalus vespertilionis* are unique amongst the British Prostriata as the length is greater than the breadth in the larval stage (Fig. 24); the length/breadth ratio at this stage in both species is about 1·41. In the larva, nymph and female of *Ceratixodes uriae* the greatest breadth of the scutum is near the front end, and the convex lateral margins meet in a rounded posterior extremity. The length/breadth ratio of the nymphal scutum is 1·46/1·0 and in the female scutum this is

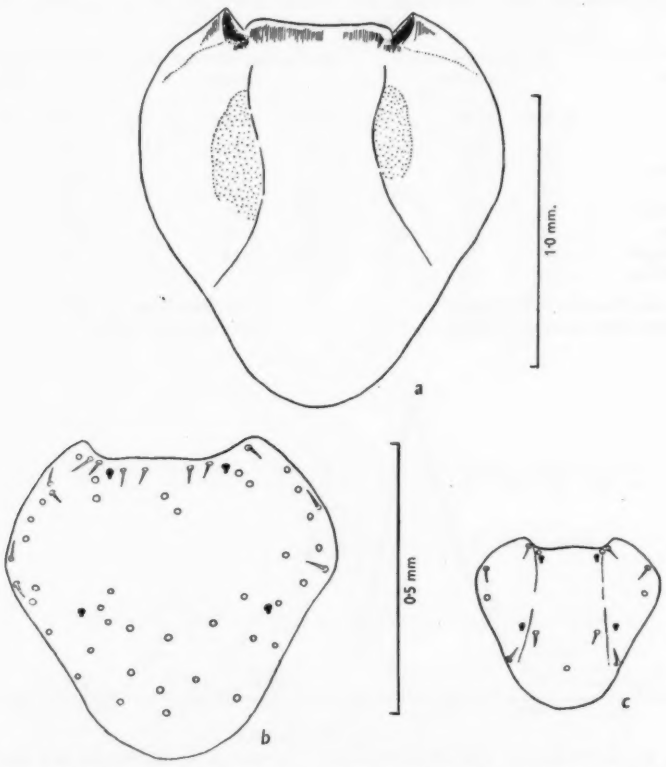


Fig. 22. *I. hexagonus*. (*a*) female scutum; (*b*) nymphal scutum; (*c*) larval scutum. Symbols in (*b*) and (*c*) as in Fig. 24.

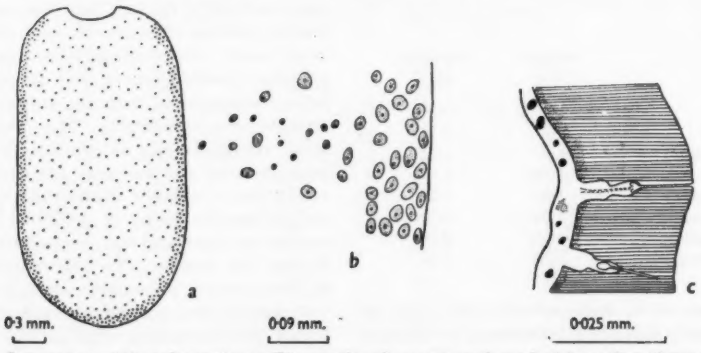


Fig. 23. *I. hexagonus*. (*a*) male scutum; (*b*) margin of scutum enlarged; (*c*) section through scutum.

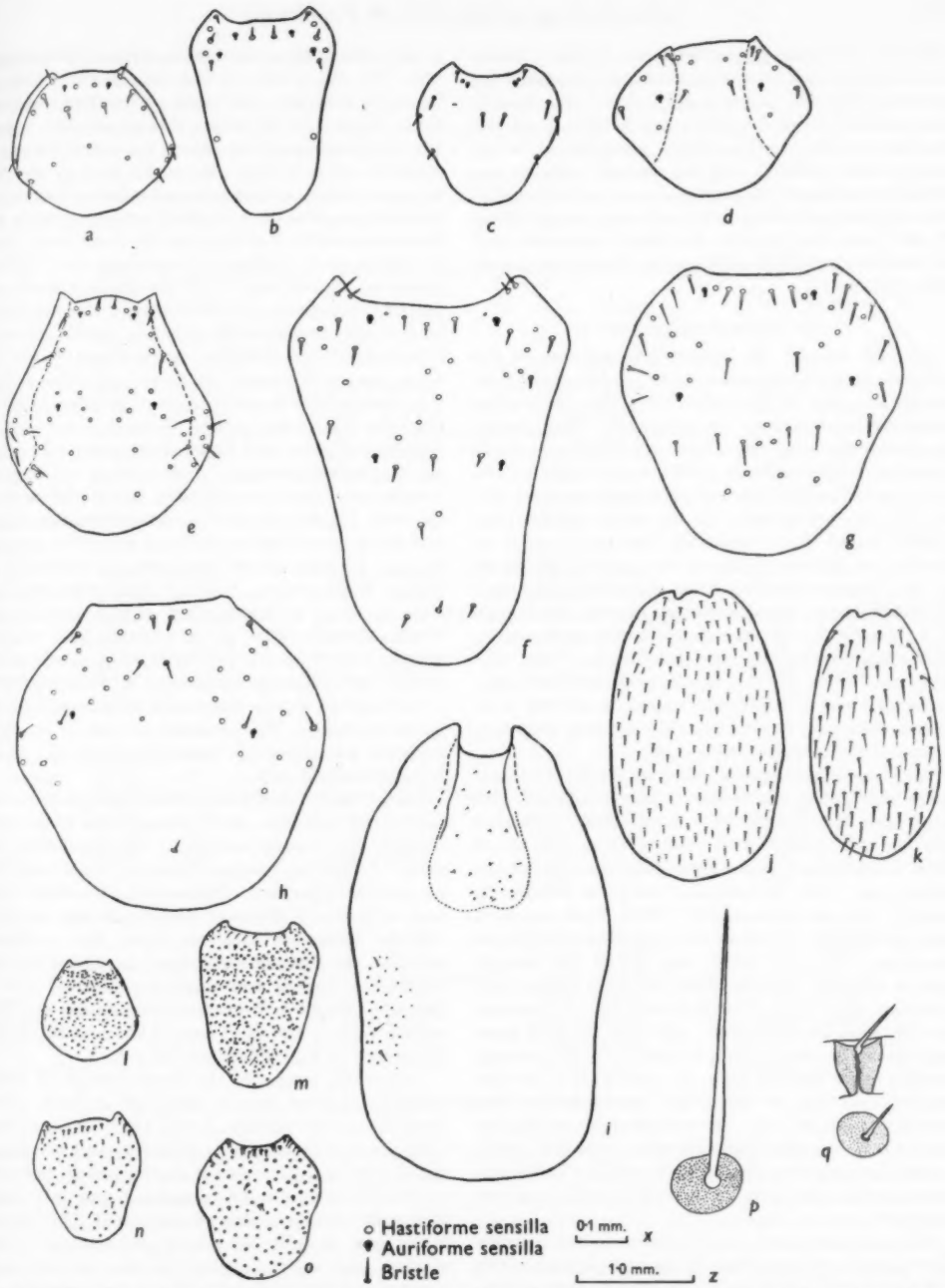


Fig. 24. The scuta of various tick species. (*a*) *I. trianguliceps*, larva; (*b*) *C. uriae*, larva; (*c*) *I. canisuga*, larva; (*d*) *I. caledonicus*, larva; (*e*) *I. trianguliceps*, nymph; (*f*) *C. uriae*, nymph; (*g*) *I. canisuga*, nymph; (*h*) *I. caledonicus*, nymph; (*i*) *C. uriae*, male; (*j*) *I. canisuga*, male; (*k*) *I. ricinus*, male; (*l*) *I. trianguliceps*, female; (*m*) *C. uriae*, female; (*n*) *I. canisuga*, female; (*o*) *I. caledonicus*, female; (*p*) scutal seta of *I. ricinus*; (*q*) scutal seta of *I. canisuga*, drawn to same scale as (*p*). (*a*)–(*h*) are drawn according to scale *X*. (*i*)–(*o*) are drawn according to scale *Z*.

1·36/1·0, so that, in contrast to *Ixodes ricinus*, *I. hexagonus* and *I. trianguliceps*, the breadth increases relatively more rapidly than the length. Long, broad cervical grooves reach back from the scapula to the postero-lateral margins in larva, nymph and female, but the lateral carinae are weakly developed. Emargination is well defined in both nymph and female, but is less so in the larva. In all stages the scapulae are bluntly rounded, and in the male they are closer together than in the female (Fig. 24*i*, *m*).

(*b*) *Cuticular structures*

(i) *Pore canals*. In balsam preparations of the scutum of *Ixodes hexagonus* and *I. canisuga* the pore canals are seen as fine striae (Fig. 23*c*), extending from the epidermis to the epicuticle. They do not penetrate the epicuticle, but end blindly in short branches. Nordenskiöld (1908) wrote that in *I. ricinus* each bundle of pore canals runs proximally into a single epidermal cell. In the same species Lees (1946) stated that 'probably the pore canals in *Ixodes* are discrete channels occupied by filaments of cytoplasm extending from the epidermal cells'. By accident the epidermis in a partially fed female of *I. hexagonus* was separated from the endocuticle. The dorso-ventral somatic musculature was also separated from it. The tapering muscle prolongations and the cytoplasmic filaments of the pore canals were seen to penetrate the scutum and stain orange with Mallory's triple stain.

(ii) *Dermal glands and cuticular ducts*. The presence of ducts in the cuticle of the female tick has long been recognized. Thus Lees (1946) indicated that in the female of *I. ricinus* there are about 1000 ducts/sq.mm. over all the alloscutum and about 400/sq.mm. over the scutum; the total number in a single tick being about 3000–7000. Their numbers and distribution in immature stages have not been described. Schulze (1942) considered the dermal glands and the cuticular ducts as sense organs and classified them into: (i) trichoid sensilla, represented by the 'sensilla auriforme', which he thought were proprioceptors responding to strains in the cuticle (unlike other sensilla they do not open on to the surface and end in expanded knobs within the cuticle); and (ii) the 'krobylophores' which included his 'sensilla sagittiformia', 'sensilla hastiformia' and the 'sensilla laterniformia', all of which are duct-like and penetrate to the surface of the cuticle.

The cuticular ducts and bristles on the scutum are fewer in the larva than in the nymph, and in the nymph than in the female. In general their distribution is fairly constant, and exhibits specific differences. I have identified the 'sensilla hastiformia' (or cuticular ducts) and 'sensilla auriforme' in the British species. Each hastiforme sensilla has a duct, whose broadened base overlies an epidermal cell. The cytoplasm of the cell extends into the lumen of this duct. The walls of the duct are thickened about midway along the length and beyond this the lumen narrows. When the alloscutal cuticle stretches after feeding, the distal part of the duct becomes wider, but this has not been observed on the scutum. The duct walls in larvae, nymphs and females are thin, but between the hard body-plates in males of *I. ricinus*, *I. canisuga* and *Eschatocephalus vespertilionis* they are thick towards the surface, becoming narrower above the epidermis. In this they resemble the ducts in *Ixodes arboricola arboricola* P.Schl. (Schulze, in Brohmer, 1944). The ducts open into concavities on the cuticular surface. The surface of each concavity in *I. canisuga* is darker than the rest of the cuticle. Similar ducts exist on the stigmal plate, and Falke (1931) referred to them as 'stigmalsinnes-organ'. According to him the distal part of the duct opened on to the surface of the spiracle. Examination of the ducts from the scutum and the spiracle fails to convince me of the presence of any internal motile elements, as described by Falke. Furthermore, I do not agree with him that they open on to the surface of the spiracle; like Nordenskiöld (1908) in *I. ricinus*, and Nuttall, Cooper & Robinson (1908) in *Haemaphysalis cinnabarina* var. *punctata* Canestrini & Fanzago, 1877, I find that in *Ixodes hexagonus* they open into the pyriform spaces. The proximal portion of the ducts contains a cytoplasmic extension from the underlying epidermal cells.

Lees (1948) stated that a certain proportion of the hastiforme sensillae in *I. ricinus* were imperfectly formed and closely resembled the auriforme sensillae of Schulze, having a normal basal canal but no external aperture. Observations on large numbers of scuta of different developmental stages of various ticks convince me that the auriforme sensillae are distinct structures, as shown by their constant numbers and relative positions. Two or four are present in immature tick stages, and they usually occupy the front part of the scutum. This is illustrated in Figs. 22*b*, *c* and 24*a–h*.

At certain stages in the development of ixodid ticks the dermal glands discharge a thick yellow 'grease' on the surface (Lees, 1947). This spreads slowly over the scutum or collects around the dermal gland opening. It is never discharged in unfed females of *I. ricinus* and *I. hexagonus*, and only begins to appear a few days after engorgement. The 'grease' is not seen in fully fed larvae and nymphs of any species, and may possibly be due to the small numbers of dermal ducts in the immature stages. It is unlikely that this secretion has a waterproofing effect, as the true waterproofing layer is laid down by the epidermal cells and is completed before moulting (Lees, 1947). Both Falke & Yalvac (1939)

suggested that it protected the cuticle and 'sense organs' from desiccation.

In my experiments, engorged nymphs and larvae which had absorbed excess water, were placed in a small chamber on a microscope stage and were observed periodically under low power. The humidity in the chamber was maintained at about 86% R.H. Droplets of water, which were not due to condensation, could be seen forming at the surface of the cuticle, but these never became very large, presumably evaporating rapidly into the humid air. In view of their general distribution over the entire scutum it seems reasonable to believe that they were not derived from the dermal glands but from the general surface of the epicuticle. The true function of the dermal glands and ducts in the Prostriata is doubtful, and the 'greasy' product in the female tick appears to add nothing of functional significance to the substance of the cuticle.

(iii) *Bristles.* On both the scutum and alloscutum the bristles are thick-walled and taper to fine points. The bristles on the scutum and also on the alloscutum are of much the same length, but in the same species the bristles on these two parts may show appreciable differences. Ten bristles, selected at random from both scutum and alloscutum of nymph, female and male ticks and all the bristles on the scutum of the larva and ten from its alloscutum were measured; the results are expressed as the ratio of length of scutal bristles/length of alloscutal bristles. (This information is summarized in Table 3.) They arise in two groups; the primaries present on the larval scutum are supplemented by secondaries after successive moults. Each bristle is set in a socket surmounting the end of a canal which penetrates the cuticle. A fine process, staining weakly in haematoxylin, can sometimes be detected within the canal. The distribution of the bristles on the scutum

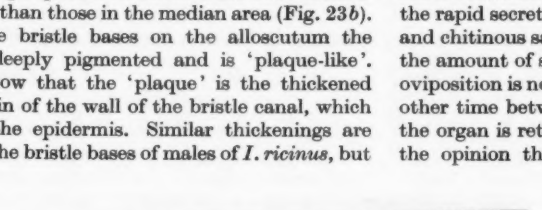
Table 5. *Mean measurements (in mm.) of the legs of* Ixodes hexagonus *Leach*

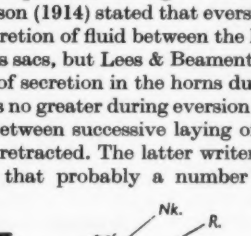
	Larva		Nymph		Male		Female	
	L	*B*	*L*	*B*	*L*	*B*	*L*	*B*
Leg I:								
Coxa	0·131	0·101	0·213	0·191	0·287	0·254	0·421	0·401
Trochanter	0·061	0·082	0·135	0·151	0·191	0·207	0·340	0·283
Femur	0·131	0·076 (0·063)*	0·243	0·143 (0·095) *	0·493	0·224 (0·127)*	0·599	0·270 (0·175)*
Tibia	0·123	0·069	0·231	0·128	0·493	0·207	0·599	0·248
Metatarsus	0·113	0·072	0·226	0·143	0·477	0·191	0·588	0·233
Tarsus	0·210	0·068	0·370	0·111	0·596	0·151	0·710	0·196
Total length	0·769	.	1·418	.	2·537	.	3·257	.
Leg II:								
Coxa	0·146	0·115	0·223	0·163	0·318	0·270	0·492	0·359
Trochanter	0·097	0·075	0·156	0·127	0·175	0·183	0·405	0·262
Femur	0·126	0·067 (0·058)*	0·218	0·119 (0·094)*	0·394	0·186 (0·127)*	0·537	0·235 (0·183)*
Tibia	0·108	0·067	0·186	0·103	0·383	0·189	0·489	0·263
Metatarsus	0·099	0·071	0·162	0·100	0·350	0·176	0·466	0·207
Tarsus	0·171	0·061	0·244	0·085	0·429	0·146	0·529	0·207
Total length	0·747	.	1·189	.	2·049	.	2·918	.
Leg III:								
Coxa	0·146	0·098	0·242	0·180	0·334	0·350	0·564	0·357
Trochanter	0·096	0·07	0·162	0·131	0·191	0·178	0·469	0·274
Femur	0·126	0·068 (0·056)*	0·236	0·113 (0·085)*	0·540	0·186 (0·127)*	0·660	0·235 (0·188)*
Tibia	0·116	0·068	0·220	0·107	0·463	0·176	0·600	0·231
Metatarsus	0·107	0·067	0·192	0·103	0·397	0·191	0·572	0·223
Tarsus	0·183	0·061	0·265	0·084	0·493	0·156	0·600	0·191
Total length	0·774	.	1·317	.	2·418	.	3·465	.
Leg IV:								
Coxa	.	.	0·233	0·176	0·350	0·366	0·575	0·399
Trochanter	.	.	0·163	0·124	0·193	0·189	0·529	0·267
Femur	.	.	0·294	0·119 (0·085)*	0·642	0·178 (0·127)*	0·853	0·226
Tibia	.	.	0·262	0·120	0·557	0·175	0·738	0·248
Metatarsus	.	.	0·240	0·120	0·477	0·175	0·639	0·239
Tarsus	.	.	0·325	0·092	0·549	0·132	0·712	0·175
Total length	.	.	1·517	.	2·768	.	4·046	.

* Width at distal end of trochanterin.

of some British species is figured in Figs. 22 and 24.

The walls of the bristle canals within the cuticle are thin in females, nymphs and larvae of all species. In males of *I. hexagonus* and *I. canisuga*, however, the bristles are close together on the edge of the scutum and inserted into deep concavities of the cuticle. The peripheral concavities are both broader and deeper than those in the median area (Fig. 23*b*). Around the bristle bases on the alloscutum the cuticle is deeply pigmented and is 'plaque-like'. Sections show that the 'plaque' is the thickened outer margin of the wall of the bristle canal, which tapers to the epidermis. Similar thickenings are present at the bristle bases of males of *I. ricinus*, but they are not as heavily pigmented, nor is the diameter of the 'plaque' as great. In males of *Ceratixodes uriae*, on the other hand, the thickening along the bristle canal walls is uniform from epicuticle to epidermis.





Figs. 25, 26.

Fig. 25. Section of Gené's organ, reconstructed.

Fig. 26. Gené's organ *in situ*.

V. GENÉ'S ORGAN

(*a*) *Review of previous literature*

This organ, first described by Gené (1848), was originally believed to serve as a receptaculum seminis. The experiments of Bertkau (1881), where eggs were prevented from touching Gené's organ, showed that such eggs shrivelled much more rapidly than eggs laid normally. Samson (1909) stated that the egg is turned to and fro for about 2 min. by the organ, and she also described the glandular structures. Lounsbury (1900) gave a description of the process in *Amblyomma hebraeum* where the organ 'unfolds its glistening arms...grasps the egg and apparently envelopes it in slime', and Wheler (1906) referred to this slime as a 'glutinous secretion'. The gland has been called an 'ovipositing' gland, the 'cephalic gland' of Christophers and the 'subscutal gland' of Samson. Lees & Beament (1948) have described the organ in *Ornithodoros moubata*, where it consists of a broad stalk surmounted by two horns, and have also shown that the gland is a proliferation of the epidermis which becomes detached from the cuticle. The mechanics of the process of eversion have not been explained with certainty, and in *Ornithodoros*, at least, it is devoid of any intrinsic musculature capable of causing its eversion. Robinson & Davidson (1914) stated that eversion is due to the rapid secretion of fluid between the hypodermal and chitinous sacs, but Lees & Beament stated that the amount of secretion in the horns during normal oviposition is no greater during eversion than at any other time between successive laying of eggs when the organ is retracted. The latter writers expressed the opinion that probably a number of indirect muscles are involved, and that the depressor muscle of the hypostome is probably the most important. Retraction of the organ of *Ornithodoros* is effected by a powerful system of retractor muscles, which penetrate between the cells of the gland and run back through the stalk to insertions on the dorsal body wall.

(*b*) *Oviposition movements*

My observations on oviposition in *Ixodes hexagonus* follow, in essential features, those made on *I. ricinus* by Lees & Beament. Each egg, on leaving the vagina of the female, is received for a few moments by the glandular organ of Gené, which is everted shortly before egg-laying commences. The organ is everted dorsally between the hind-margin of the basis capituli and the front end of the scutum. It is absent from the male and the immature stages of ticks and all other acarine groups.

(*c*) *Structure of the organ*

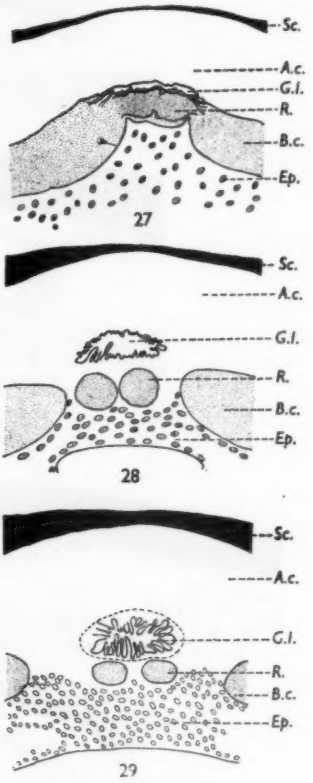
The organ in *I. hexagonus* consists of a broad stalk surmounted, as in all *Ixodes* ticks, by four horns (Figs. 25, 26). The organ is about 640 μ long, and from the external opening to the base of the horns is about 320 μ. The horns are prominent and usually

contain a highly refractile liquid, which occupies the space between the epidermis and the cuticle. The quantity of liquid in the horns may vary and sometimes there is only a little at the tips.

The opening of the organ lies above the dorsal posterior margin of the basis capituli and below the anterior edge of the scutum. The aperture is surrounded by an intucking of the alloscutal endocuticle, which at first is closely applied to the lower surface of the scutum. The cuticle of Gené's organ includes epicuticle and endocuticle, and passes without interruption into the cuticular layers of the body wall. A transverse pigmented 'bar' lies below the aperture, and is fused with the posterior dorsal surface of the basis capituli (Fig. 27). The epidermis is thickened beneath the bar, and is partially derived from the epidermal layer underlying the cuticle of the basis and partially from the investing layer of the inner cheliceral sheath (Fig. 27).

The transverse bar, seen only on a level with the basis, is formed by the fusion of two converging pigmented rods. These lie beneath the lumen of the gland and pass back for about 210–220 μ, i.e. they are limited to the proximal part of the stalk. Anteriorly they are close together but posteriorly diverge, becoming smaller in diameter. For about the first 50 μ they are embedded in cuticle (Figs. 28, 29), but thereafter become gradually ensheathed by epidermal cells which spread from the mesial and lateral aspects (Fig. 30). Farther back the cell-layer becomes thicker and forms a conspicuous median ridge (Figs. 32, 33), and at the end of the rods the ridge nearly divides the lumen of the stalk into equal halves. This division is actually accomplished at about 320–340 μ from the proximal end by the backward passing muscles (Figs. 34). These muscles are attached to the ends of each rod and inserted on each side of the mid-line of the scutum, and at some 800 μ from the front edge of the latter.

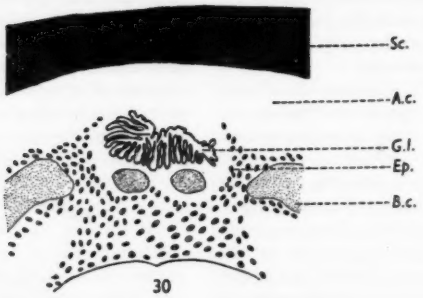
Before the stalk bifurcates into the horns, it lies above the chelicerae (Figs. 27–34), but the horns themselves are lateral to these structures (Figs. 36, 37). At the point of bifurcation the horns are single structures (Fig. 35) with a distinctive cuticular lining. Laterally this cuticle is thrown into deep, closely set ridges; on the ventral surface, except for one prominent folded protuberance, the ridges are lower and set far apart, and from the dorsal surface a large, longitudinal fold of cuticle hangs into the lumen of the organ. Slightly farther back (Fig. 36) this fold lengthens, and ultimately fuses with the ventral prominence. Thus the horns become bifid. The cuticular fold is filled with cells from each projection, and accordingly the cuticle of both horns is surrounded by a single layer of epidermal cells (Figs. 36, 37). This cuticle does not extend for the entire length of the horns (Fig. 25) so that a space exists between the cuticle and the epidermis. In newly metamorphosed female ticks, both cuticle and epidermis lie close against each other, except for this space, but when the tick is ready to lay eggs the cuticle and epidermis separate. The entire organ then consists of a layer of cuticle surrounded by a layer of opisthosomatic epidermis. In this respect it agrees with *Argas persicus* as described by Robinson & Davidson (1913–14).



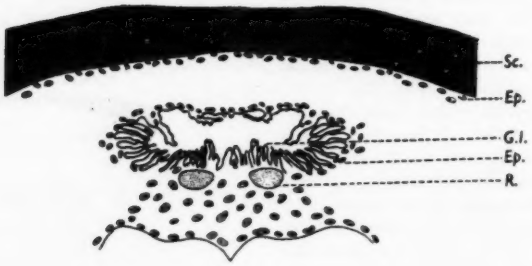
Figs. 27–29.

Figs. 27–37. Transverse sections through Gené's organ. For explanations see text.

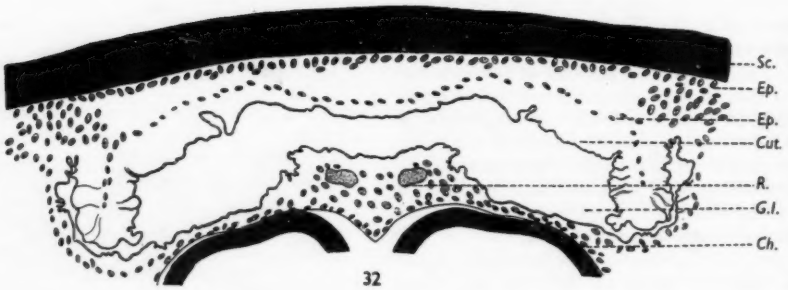
The glandular portion is a specialized region of the epidermis. At the base of the stalk the epidermal cells are relatively attenuated, but near the base of the horns, at about 110 μ from the opening of the organ, a thick, folded epidermis forms the gland (Fig. 33). Histologically this glandular portion is distinguished by the columnar or wedge-shaped cells, with well-defined boundaries, and nuclei which lie near the margin of the cell and abut against the haemocoele. The gland is 60–70 μ long and is located above and beside the much folded cuticle of the stalk-lumen. Cytoplasmic extensions pass from the



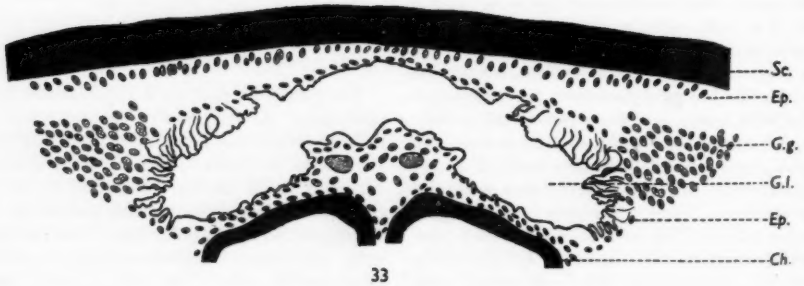
30



31

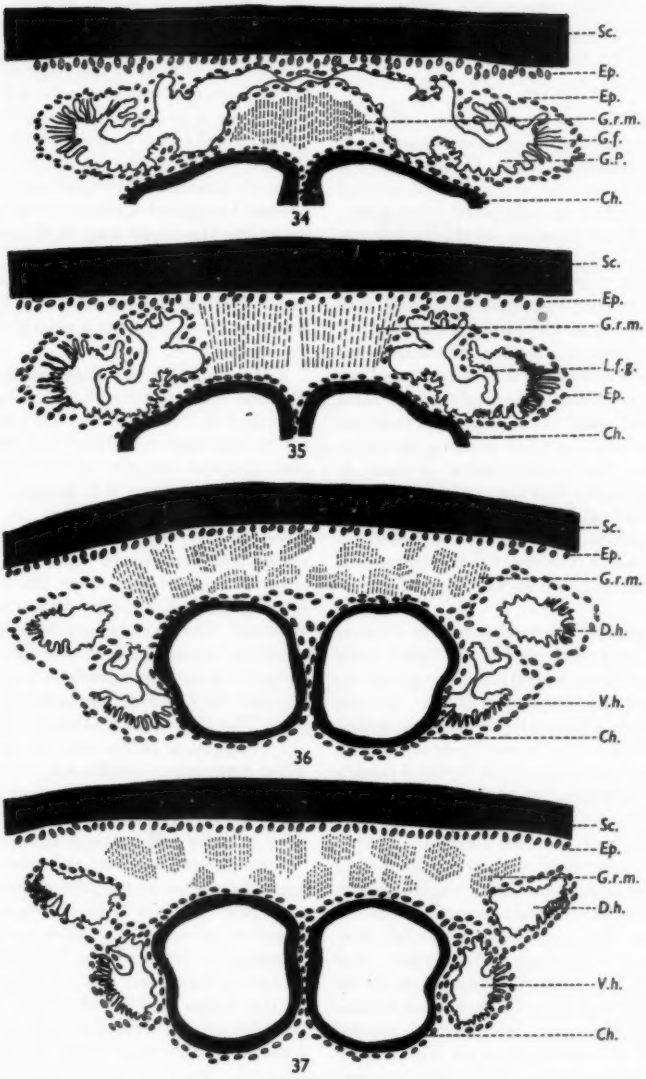


32



33

Figs. 30–33.



Figs. 34–37.

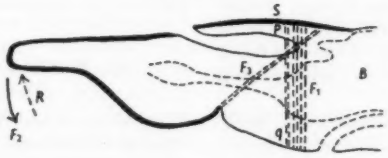


Fig. 37A. Diagram to show possible factors involved in everting Gené's organ.

gland through the folded cuticle and presumably open into the lumen of the gland. The gland does not extend into the horns.

(*d*) *Eversion*

The mechanics of the eversion process have not been made out with certainty. In *Ixodes hexagonus*, *I. canisuga* and *I. ricinus*, at least, Gené's organ is without any intrinsic musculature capable of causing eversion. Consequently a knowledge of other structures, particularly of the muscles, in the podosoma is necessary.

Observations show that the capitulum describes a vertical arc before the eggs are laid, and this movement is effected, in part at least, by contraction of the capitulum depressor muscles. These muscles originate on the lower surface of the scutum, and are inserted on the posterior ventral margin of the basis capituli. At the same time as the depressor muscles contract, the palpal muscles relax, causing the palps to splay outwards. The observations of Lees & Beament and myself agree that the capitulum comes to lie against the ventral body wall just in front of the vagina. This cannot be completely effected by the capitulum depressor muscles, for the latter will only pull the capitulum through an angle of 90° at most.

The vagina, located in the podosoma, is divided into a posterior or cervical region, provided with a thick investment of muscles and lined by epicuticle, and a short vestibular region without circular muscles but lined with a highly folded epicuticle and thin-layered endocuticle.The dorso-ventral somatic muscles start at the bifurcation of the horns of Gené's organ and extend back along the body. At the level of the vagina the dorso-ventral muscles are closely linked with the vaginal muscles (Arthur, 1951*b*) and striped fibres pass out from the vertical ones to the walls of the cervical portion of the vagina. From the postgenital area of the ventral body wall,retractor genital muscles pass obliquely forwards to the posterior lip of the genital aperture in the female. The vestibular part of the vagina during egg-laying begins to unroll like the finger of a glove and is completely evaginated through the external genital opening in the form of a short ovipositor with a truncated extremity. Probably contractions of the circular muscles of the cervix are responsible for the prolapse of the vagina during egg-laying. Subsequently, the eggs pass into the vestibular portion of the ovipositor, the final extrusion of the egg being brought about by contraction of the dorso-ventral muscles.

Since the sclerotized rods are, structurally, backward extensions of the basis capituli, any downward movement of the latter will initiate the eversion of Gené's organ. It seems improbable that this will evert the organ completely, but it will be adequate to overcome the initial inertia. Lees & Beament wrote that in *Ornithodoros* the organ can be everted with a much smaller application of pressure if the hypostome is held in the depressed position. Similarly, if the capitulum of a fully fed female of *Ixodes hexagonus* is depressed the application of a smaller pressure will cause eversion, which suggests that the flexure of the capitulum is the prelude to eversion.

In the absence of intrinsic muscles to complete the process I suggest that once the initial inertia has been overcome the organ may be forced out mechanically in the following way (Fig. 37 A). The basis moves in the direction of F_2, and contraction of muscle *pq* will cause further movement of the capitulum downwards as there is no force acting on the capitulum in the direction *R*.

There will also be a diagonal reaction in direction F_3. The combination of forces F_1 and F_3 will have no outlet in the direction of the mass of the body *B*, for at this stage it is distended with blood. They are also opposed dorsally by the hard scutal plate *S*. The resultant force will therefore be exerted along the line of least resistance, i.e. on Gené's organ. Accordingly, such upward muscular contraction acting simultaneously with the movement of the capitulum downwards has the effect of a squeeze, forcing the hollow organ outwards and reversing the surfaces. This is comparable to the 'squeegee' toy, where an invaginated rubber figure is enclosed within another stouter rubber covering, and the object everted by external pressure on the outer rubber case. The same principle might apply in the tick, the only difference being that the pressure is now applied internally. Additional forces are also brought to bear on the organ, for when the capitulum curves, the posterior portions of the chelicerae and associated muscles will impinge against the organ.

I have not observed the powerful system of retractor muscles which penetrate between the cells of the gland and run back through the stalk to insertions on the body wall, such as observed by Lees & Beament in *Ornithodoros*. The only retractor muscles in *Ixodes hexagonus* are attached at the bifurcation of the horns and on the apex of each horn. The muscles arising from the rods are not direct retractors of Gené's organ, but retractors of the basis capituli. But obviously, since the rods are closely associated with the intucked cuticle of Gené's organ, they are of importance in assisting its retraction.

VI. MECHANISM OF STRETCHING DURING FEEDING

Comparison of unfed and fully fed ticks shows a very substantial increase in size; thus unfed and fully fed females weigh 1·8 and 250 mg. respectively, i.e. a weight increase of about 140 times. The cuticle of the opisthosoma must therefore stretch consider-

ably, and this is partly accomplished by the flattening of the epicuticular folds. These folds resemble finger print impressions in all stages of *I. canisuga*, *I. hexagonus*, *I. ricinus*, *I. trianguliceps*, *I. unicavatus* and *Eschatocephalus vespertilionis*, but in *Ceratixodes uriae* they appear like the 'skin' of scalded milk.

The unfed larva is flattened dorso-ventrally (Fig. 38*a*) and the epicuticle thrown into deep folds. When the opisthosoma is distended with blood the endocuticle is greatly stretched and attenuated, and the folds of the epicuticle become smoothed out. This mechanism allows for the enormous change in volume and for the fully fed larva to become rounded in transverse section. Presumably this is adequate in the larva where the cuticle is thin, as indicated by its cutaneous method of breathing. In later stages, however, the cuticle is thicker and tougher, and tracheal breathing takes place.

The epicuticle in the nymph and female is also thrown into folds, but in addition there are longitudinal folds of the cuticle along the sides and lower surface of the body. These lie above the insertions of the coxae (Fig. 38*b*) and pass postero-ventrally, coming to lie below the spiracles (Fig. 38*c*). The folds become less pronounced posteriorly and cease on reaching the anus, but a smaller fold lies nearer the midline (Fig. 38*d*) which persists to the hind-margin. As in the larva the endocuticle is plastic and during feeding becomes extended with the consequent flattening of the epicuticular folds. The body folds likewise become stretched but to an even greater extent than the epicuticular folds.

Unlike the larva, nymph and female, the opisthosoma of the male is almost completely invested by hard, rigid plates. The cuticle between the plates is flexible, thin and folded, thus permitting the volume

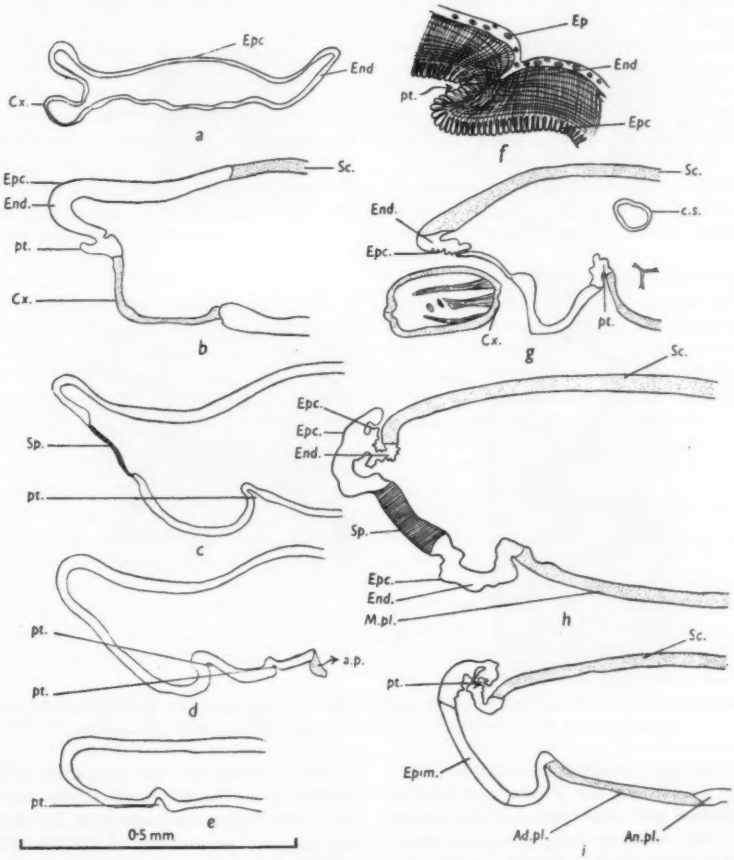


Fig. 38. Methods of stretching of the cuticle by different tick stages. For explanation see text. (*a*) larva; (*b*)–(*e*) female; (*g*)–(*i*) male to show position of the body folds; (*f*) high power drawing of fold indicated in (*b*).

of the body to increase. The main fold commences at the level of the pseudoscutum (Fig. 38*g*) and is dorsal to the insertion of the legs. It continues back above the spiracle and thence to the hind-margin of the scutum. The outer wall of this fold has a smooth epicuticle, like the hard plates, but along the inner edge the epicuticle is much folded (Fig. 38*h*). A less pronounced folding exists on the lower surface of the body, between the epimeral and the median plates.

Between the valves and annulus there is a flexible cuticle which permits the valves to open and close. The major axis of the adult anus is longitudinal, but in larvae and nymphs the longitudinal and transverse axes are about equal. In many larvae and nymphs, indeed, the transverse axis may sometimes be even slightly longer. Dorso-ventral body muscles are attached to the inner surface of each valve, and on the outer surface four bristles occur.

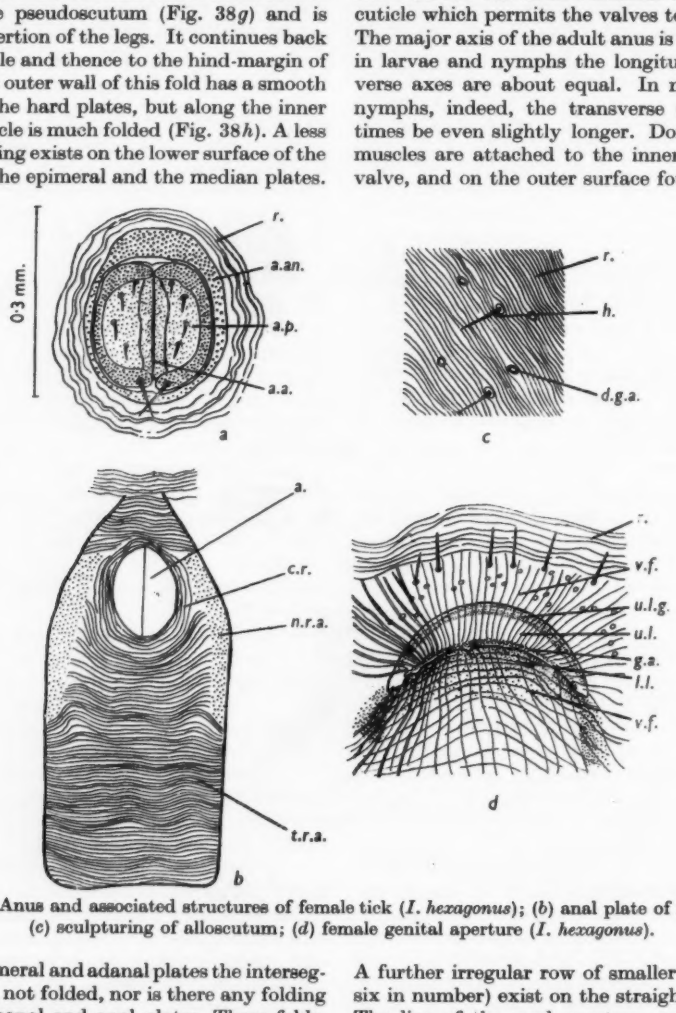


Fig. 39. (*a*) Anus and associated structures of female tick (*I. hexagonus*); (*b*) anal plate of *I. hexagonus*; (*c*) sculpturing of alloscutum; (*d*) female genital aperture (*I. hexagonus*).

Between the epimeral and adanal plates the intersegmental cuticle is not folded, nor is there any folding between the adnanal and anal plates. These folds, coupled with the large cheliceral digits, suggest that, morphologically at least, there is nothing to prevent males of *Ixodes hexagonus* from feeding.

VII. ALLOSCUTAL ORIFICES AND APPENDAGES

(*a*) *The anus*

In *I. hexagonus* the anus lies on the mid-ventral surface and slightly behind the middle of the body (Fig. 39*a*). It is a slit-like aperture bordered by two hard, thick semicircular plates or valves, and surrounded by a thickened ridge of cuticle, the annulus. A further irregular row of smaller bristles (usually six in number) exist on the straight mesial margin. The lips of the anal aperture are formed by the slightly prolapsed folds of cuticle which line the rectal sac and these are usually visible between the valves.

(*b*) *Genital opening*

In both sexes the genital opening (Fig. 39*d*) is also on the mid-ventral surface and level with the second intercoxal space. The position of the genital opening in the female varies according to the degree of body distension. Thus when fully fed it lies opposite the third intercoxal space.

The female aperture is a crescentic slit bordered by tumid lips. The posterior (or lower) lip is more tumid than the anterior (or upper), and during egg

laying the inner lining of the former rolls forward to form the 'ovipositor', so that the opening is directed towards the depressed capitulum, an arrangement admirably adapted to transfer the egg to Gené's organ. The lower lip is continued into the cuticle of the opisthosoma, and this stretches sufficiently to allow the passage of the eggs. The cuticle is reflexed into the genital orifice and forms the chitinous intima of the vagina. It is this portion which is evaginated through the orifice during egg laying. Injecting fluids into the body brings about the same result. The front margin of the upper lip bears a groove parallel with its front edge.

The male opening is smaller than the female, but more strongly arched with the convexity towards the basis capituli and an intromittent organ is absent.

The larva and nymph have no genital openings, but before the fully fed nymph metamorphoses a small, imperforate, dimple-like depression is seen between the second and third coxae.

(*c*) *The legs*

As in other Prostriata the nymphs and adults of *I. hexagonus* have four pairs of walking legs, but in the larva the hindermost pair are suppressed. The legs are longer than in Argasid ticks, and when compared with other species of the Prostriata are moderate in length (Table 5). They are attached to the antero-ventral surface of the body, and at rest the first two pairs point forward and the two hindermost pairs backward. In the living animal the legs are pale brown with narrow bands of unpigmented cuticle at the junction of the segments with the flexible articular membranes. Each leg has six joints, and from the proximal to the distal end these are the coxa, trochanter, femur, tibia (patella of Falke, 1931), metatarsus (tibia of Falke) and tarsus. The tarsus is connected to the pulvillus by the caruncle.

The coxae fit into apertures on the ventral body wall, and by adduction and abduction a little movement is possible. The articulation of coxa and trochanter permits only movement parallel to the surface; between the trochanter and femur there is a universal joint. The most distal segments move only dorso-ventrally.

The basal portion of the femur on all legs is separated from the remainder of the segment by a joint-like constriction, forming a pseudo-article, the trochanterin of Lahille, and bears a trochanterin organ. The trochanterin is of about equal length in all the legs. A similar pseudo-article, the pseudo-tarsus, is present on the proximal end of each tarsus, and in the foreleg is distinguished as a constriction at the basal end of the tarsus. The pseudo-tarsus increases in length from the first to the fourth legs.

During progression the first pair of legs in the nymph and the adult are out of phase with the remaining three pairs. The forelegs may be held out rigidly in front of the body and move parallel with the ground, when articulation is between the coxa and trochanter. Alternatively, they may wave actively in the same way as the antennae of insects, and articulation is then between trochanter and femur. Fed ticks use the first method only, but unfed ticks employ both means. As the second pair of legs moves forward the third pair comes to occupy the original position of the second pair. The fourth pair of legs moves forward later than the third pair and because they are always outstretched and move parallel to the surface they encompass a wide arc. The forelegs in the hexapod larvae move in the same way as the second pair of the nymphs and adults; the second and third pairs of larval legs progress in a similar manner to the third and fourth pairs of these stages. Since the forelimbs in unfed stages are also responsible for questing, larvae obviously cannot progress and quest at one and the same time.

The lengths of the individual segments of the legs were measured along their upper surfaces, and the breadths (other than that of the tarsus) across their apices. The breadth of the tarsus was measured from the apical hump to an infolding on the lower surface. These data are summarized in Table 5 and in Fig. 40.

Analysis of these data show that:

(*a*) There is a general resemblance in the length of individual segments of the legs of all developmental stages (Fig. 40).

(*b*) The total lengths of the segments (henceforth referred to as 'leg-length') decrease in the following order in the larva: III, I, II; in the nymph: IV, I, III, II; in the male: IV, I, III, II; and in the female: IV, III, I, II.

(*c*) During metamorphosis of the nymph, the 'leg-length' of the female increases more rapidly than that of the male, and the shortest 'leg-length' in the female (II) exceeds the longest 'leg-length' in the male (IV).

(*d*) The trochanter is the shortest segment in the legs of all developmental stages; the tarsus is the longest segment in all the legs of the larva and nymph, in legs I and II of the male and leg I of the female; the femur is longest in legs III and IV of the male, and in legs II, III and IV of the female.

(*e*) The mean percentage increase in the length of any one segment from stage to stage varies, i.e. the growth rates of the individual segments of the legs are not uniform. This is further shown in Table 6.

(*f*) Distally the tarsi taper, and in Table 5 the breadth has been measured across the hump. In all other segments the greatest width is at the apex.

Lees (1948) observed thick-walled bristles on the legs of *I. ricinus* and ascribed a tactile function to them. Similar bristles are found on the legs of *I. hexagonus* and these point towards the pulvillus.

On the three distal segments of the leg they are confined to the ventral surface, but on the proximal segments they also occur on the lateral and dorsal surfaces. Some sixty to seventy-five tactile bristles

VIII. SUMMARY

The palps of all stages of *Ixodes trianguliceps* are provided with a ventral plate below the basal article;

Table 6. *Mean percentage increase in 'leg length' from stage to stage*

Segment	Leg	Mean percentage increase: Larva to nymph	Nymph to male	Nymph to female
Coxa	I	62·6	34·7	97·7
	II	52·7	42·6	120·5
	III	65·8	38·0	133·0
	IV	—	50·2	146·8
Trochanter	I	121·3	41·5	151·8
	II	60·8	12·1	159·9
	III	68·7	17·9	189·5
	IV	—	18·4	224·5
Femur	I	85·5	102·9	146·5
	II	73·0	80·7	146·3
	III	87·3	128·8	179·6
	IV	—	118·3	190·1
Tibia	I	87·8	113·4	159·3
	II	72·2	105·9	162·9
	III	89·6	110·4	172·7
	IV	—	112·6	181·6
Metatarsus	I	100	111·0	160·1
	II	63·6	116·4	187·6
	III	79·4	106·7	197·9
	IV	—	98·7	166·2
Tarsus	I	76·1	61·0	91·9
	II	42·7	71·7	116·7
	III	44·8	86·0	126·4
	IV	—	68·9	119

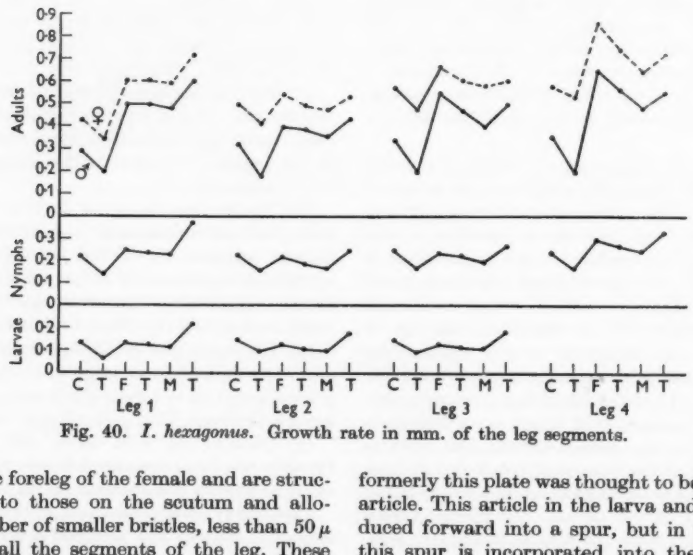


Fig. 40. *I. hexagonus*. Growth rate in mm. of the leg segments.

are found on the foreleg of the female and are structurally similar to those on the scutum and alloscutum. A number of smaller bristles, less than 50 μ long, occur on all the segments of the leg. These bristles are limited to the dorsal and lateral surfaces.

formerly this plate was thought to be the first palpal article. This article in the larva and nymph is produced forward into a spur, but in the female tick this spur is incorporated into the basis capituli as the sella. The hypostomes of *I. trianguliceps* and

I. canisuga are redescribed to clear up existing inaccuracies.

Ticks in which the rostrum does not extend beyond the apex of the first palpal article are found on birds, and this probably represents a primitive condition. Those with palpal spurs, which may or may not be fused with the basis capituli, are found on birds (not in Britain) or on mammals of the mouse size group, and those where the rostrum is produced beyond the first palpal article occur on a wide range of large and small animals. The longer and more heavily toothed digits of ticks appear to be associated with a wide host range and vice versa. The structure of the digit may also influence the choice of attachment sites by ticks on their hosts because the microstructure of the skin varies in different parts.

Variations in size and form of the scuta of some British ticks are described, and the mean growth rate is ascertained from this data. The information shows that the material of *I. ricinus* and *I. hexagonus* is homogeneous, and that specific differences occur in size, shape, the position and type of dermal ducts and in the relation between scutal and alloscutal bristles.

The morphology of Gené's organ in *I. hexagonus* is described. It consists of a basal sac-like portion surmounted by four horns and lined with a cuticle beneath an epithelium. The gland is a proliferation of the epithelium and located near the bifurcation of the base into the horns. A watery refractile fluid, secreted by the gland, accumulates between the epidermis and the cuticle in the horn-like extensions. Proximally the thick endocuticle and epidermis lie close together, and two cuticularized rods penetrate the endocuticle for about half-way along the stalk. The rods arise from the postero-dorsal margin of the basis capituli. Muscles, arising from the free ends of the rods, pass back to near the hind-margin of the scutum; they retract the basis capituli after egg laying and indirectly assist in the retraction of Gené's organ. A suggested mechanism for everting Gené's organ in *I. hexagonus* is outlined.

During feeding the opisthosomatic cuticle of all developmental stages of the tick is much stretched. In the larva, where the cuticle is thin, this is effected by the flattening of the epicuticular pleats, but nymphs and females have, in addition, two longitudinal folds alongside the body which stretch to a far greater extent than do the epicuticular pleats. Similar folds are present between the hard ventral plates and the scutum and between the epimeral and median plates in the male. Coupled with the large size of the male digit, this suggests that morphologically, at least, males can imbibe blood.

The growth of the leg segments of *I. hexagonus* is not constant from stage to stage, and as a result the shortest 'leg-length' of the female exceeds the longest 'leg-length' of the male.

REFERENCES

ARTHUR, D. R. (1946). *Parasitology*, **37**, 154.
ARTHUR, D. R. (1949). *Parasitology*, **39**, 235.
ARTHUR, D. R. (1951*a*). *Parasitology*, **41**, 66.
ARTHUR, D. R. (1951*b*). *Parasitology*, **41**, 82.
BERTKAU, P. (1881). *Verh. naturh. Ver. preuss. Rheinl. J.* **38**, 145.
BERNSHTEIN, M. M. (1948). *Legkaya. Prom.* **5**, 18.
BRAUN, REISMANN, IVANOV & RJABINEN (1932). *Centraini naucoissledovatelksi koz promyslennosti*. Leningrad.
BROHMER, P. (1944). *Fauna der Deutschland*, pp. 453–8. Berlin.
CARSTENS, P. & KINZELBACH, W. (1933). *Züchtungskunde*, **8**, 135.
CHIODI, V. (1933). *Mont. ital. zool.* **43**, 284.
ELTON, C. & KEAY, G. (1936). *Parasitology*, **28**, 110.
FALKE, H. (1931). *Z. Morph. Ökol. Tiere*, **21**, 567.
GENÉ, J. (1848). *Mem. R. Accad. Sci. Torino*, **9**, 751.
GREGSON, J. D. (1937). *Proc. Ent. Soc. B.C.* **33**, 15.
HUGHES, T. E. (1949). *Ann. Trop. Med. Parasit.* **43**, 349.
JONES, B. M. (1950). *Parasitology*, **40**, 247.
LEES, A. D. (1946). *Parasitology*, **37**, 1.
LEES, A. D. (1947). *J. Exp. Biol.* **23**, 379.
LEES, A. D. (1948). *J. Exp. Biol.* **25**, 145.
LEES, A. D. & BEAMENT, J. W. L. (1948). *Quart. J. Micr. Soc.* **89**, 291.
LOUNSBURY, C. P. (1900). *Ent. News*, **11**, 326.
MILNE, A. (1947). *Parasitology*, **38**, 34.
NEMEC, V. (1940). *J. Int. Soc. Leath. Chem.* **24**, 243.
NEUMANN, L. (1899). *Mém. Soc. zool.* **12**, 129.
NORDENSKIÖLD, E. (1908). *Zool. Jb.* Abt. 2, **25**, 637.
NUTTALL, G. H. F., COOPER, W. F. & ROBINSON, L. E. (1908). *Parasitology*, **1**, 238.
NUTTALL, G. H. F., WARBURTON, C., COOPER, W. E. & ROBINSON, L. E. (1911). *Ticks. A monograph of the Ixodoidea*, Pt. II. Cambridge University Press.
ROBINSON, L. E. & DAVIDSON, J. (1913–14). *Parasitology*, **6**, 20, 217, 382.
SAMSON, K. (1909). *Z. wiss. Zool.* **93**, 185.
SCHULZE, P. (1942). *Z. Morph. Ökol. Tiere*, **38**, 379.
SNODGRASS, R. E. (1948). The feeding organs of Arachnida, including mites and ticks. *Smithsonian Misc. Coll.* **110**, no. 10. Washington, D.C.
TALICE, R. V. (1930). *Ann. Parasit. hum. comp.* **8**, 173.
WHELER, E. G. (1899). *Proc. R. Agric. Soc. Engl.* **4**, 626.
WHELER, E. G. (1906). *J. Agric. Sci.* **1**, 400.
WITH, C. J. (1904). *Vidensk. Medd. naturh. Foren. Kbh.* **56**, 137.
YALVAC, S. (1939). *Z. Morph. Ökol. Tiere*, **35**, 535.

Abbreviations used in the figures

A.	anterior.
A.c.	alloscutal cuticle.
An.pl.	anal plate.
Ad.pl.	adanal plate.
a.	anus.
a.a.	anal aperture.
a.an.	annulus.
a.p.	anal valve.
a.p. i	anterior projection of palpal article i
a.po.	porose area.
B.	body of Gené's organ.
B.c.	basis capituli.
B.s.	bulbous base of chelicera.
Ch.	chelicera.
Ch.S.	inner cheliceral sheath.
Cut.	cuticle.
Cx.	coxa.
c.	cornua.
c.r.	concentric ridges.
c.s.	cheliceral shaft.
D.	dorsal.
D.h.	dorsal horn of Gené's organ.
D.p.	dorsal process.
D.r.m.	dorsal horn retractor muscle.
d.	digit.
d.g.a.	dermal duct aperture.
d.r.	dorsal ridge.
E.a.	external article.
E.a.t.	teeth of external article.
Ep.	epidermis.
Epc.	epicuticle.
Epim.	epimeral plate.
End.	endocuticle.
G.g.	gland of Gené's organ.
G.f.	cuticular folds of Gené's organ.
G.l.	lumen of Gené's organ.
G.r.m.	retractor muscles of basis capituli.
g.a.	genital aperture.
H.	hypostome.
H.r.m.	retractor muscle of the organ.
h.	bristle.
I.a.	internal article.
L.f.g.	longitudinal fold of Gené's organ.
l.l.	lower lip of vaginal orifice.
M.pl.	median plate.
Nk.	neck of Gené's organ.
n.r.a.	non-ridged alloscutum.
p.	palp.
p. i–iv.	palpal articles i to iv.
p.p. i	ventral plate of palpal article i.
pt.	pleat.
R.	rods of Gené's organ.
Ro.	rostrum.
r.	alloscutal ridges.
Sc.	scutum.
Sp.	spiracle.
s.	sensilla.
t.r.a.	transverse ridges of alloscutum.
u.l.	upper lip of vagina.
u.l.g.	groove on upper lip.
V.	ventral portion of basis capituli.
V.h.	ventral horn of Gené's organ.
V.r.m.	ventral horn retractor muscle.
v.f.	vaginal folds.

(*MS. received for publication* 12. x. 1951.—Ed.)

THE CAPITULUM AND FEEDING MECHANISM OF *IXODES HEXAGONUS* LEACH. II

By D. R. ARTHUR *Department of Zoology, King's College, University of London*

(With 7 Figures in the Text)

1. INTRODUCTION

The capitulum of the tick *Ixodes hexagonus* Leach has been described (Arthur, 1951) and consists of an extra-basis and basis regions. Usually only the extra-basis region is embedded in the host's skin. A food channel, which is divided into three sections, runs through these two parts. From the distal to the proximal ends these sections are (i) the hypostomal groove, which is also the distal portion of the salivary channel; (ii) the labro-hypostomal groove; and (iii) the labro-hypostomal gutter, both of which are covered by the labrum. The hypostomal groove is roofed over by the chelicerae and the latter also overlie the labrum, thus forming a roof to the salivary channel.

The relationship of the component parts of the extra-basis region during feeding can best be understood by the examination of the mouthparts when they are inserted in the host's skin. By this method the histo-pathological changes of the host tissue caused by the tick bite can also be ascertained. Hoeppli & Feng (1931) have investigated the action of the bite of *Dermacentor sinicus* P.Sch. and *Haemaphysalis campanulata hoeppliana* P.Sch, Cowdry & Danks (1933) that of *Rhipicephalus appendiculatus*, and Moskwin (1929) those of *Ornithodoros papillipes* and *O. tholozani*. Their results showed that in animals carrying natural infestations of ticks a very extensive haemorrhage was always observed in the centre of the lesions which they caused. Pawlowsky & Stein (1927) studied the histological changes in the human skin after injections of emulsions from the salivary glands of *Ixodes ricinus* L. but no haemorrhage was evident after such artificial injections. Apart from these there are but few records of the histological study of actual lesions caused by these parasites.

2. METHODS AND MATERIALS

The present observations were made on ticks attached to hedgehogs and stoats. These were obtained from Wye and Ammanford through the courtesy of Mr M. D. Froud, M.Sc., and Mr William Thomas. On receipt in the laboratory the host animals were chloroformed and the posterior parts of the feeding ticks were cut away to prevent withdrawal of the rostrum. The skin around the attached ticks was then excised, fixed in Bouin's alcoholic fixative and embedded in paraffin wax (m.p. 58° C.). Serial sections were cut and stained in either haematoxylin-eosin or Mallory's triple stain. The drawings of the mouthparts *in situ* were made with the aid of the camera lucida, under an oil-immersion lens, and no attempt at schematization of the figures has been made.

3. METHOD OF PENETRATION

The technique employed to observe the behaviour of the tick during the process of penetration was similar to that used by Lees (1948, p. 193) in his membrane experiments. Freshly removed skin from the perineum of a hedgehog was used for membranes, the hair was cut short to facilitate observation, and the apparatus was kept at 32° C. Before penetrating the freshly prepared skin membrane the tick draws back its palps and appears to be partially supported on the extra-basis region. The chelicerae appear to be extruded by contraction of the dorso-ventral muscles of the opisthosoma and the digit thus touches the host's skin. Movement of the legs causes the body to tilt upwards at an angle of about 45° and the mouthparts begin to penetrate the skin. The tilting of the body, which may be increased as embedding proceeds, suggests that it assists the internal article of the digit to make the first cut. A forward movement of the body follows and the digits penetrate the host tissue by their shearing action. The hypostome is then raised and inserted into the wound. This is probably effected by the contraction of the elevator muscles of the hypostome (see p. 188). The initial embedding is a gradual process, with the hypostome and chelicerae being forced deeper into the skin, within which further laceration of the tissue probably takes place. In these membrane experiments the mouthparts of *I. hexagonus* were completely embedded in 7–13 min. Ten unfed females became attached in 9, 12, 12, 13, 10, 12, 11, 7, 13 and 8 min. after the preliminary 'balancing' act. The mouthparts of seven unfed females failed to penetrate the membranes after 24 hr. The extra-basis region may enter the skin vertically, as is usual with female ticks, or it may

penetrate at an angle. This is seen particularly with nymphs when they become attached in the groin region (Fig. 1).

hypostomal canal. The teeth protrude laterally and ventro-laterally, and their cavities are continuous with those of the chambers. These are lined by

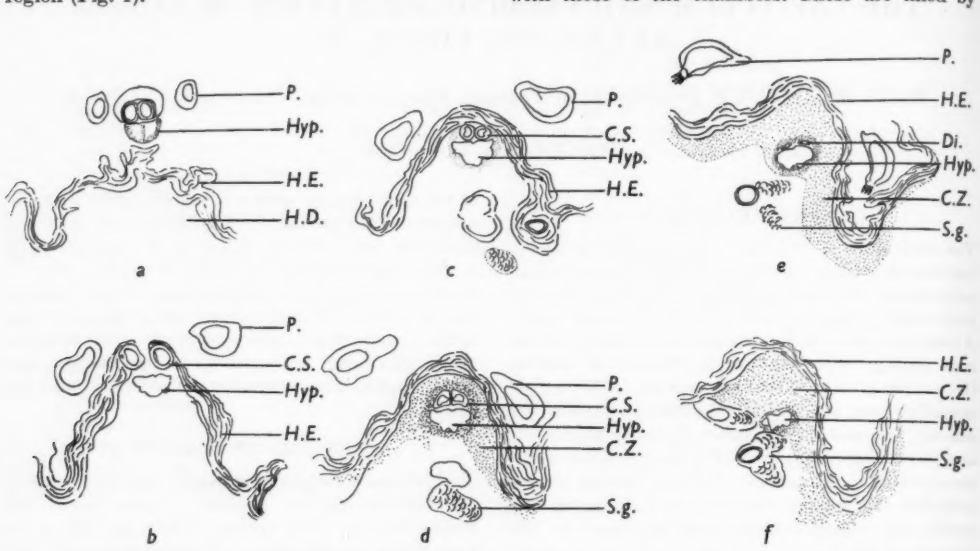


Fig. 1. Transverse sections of the skin of a hedgehog to show penetration by the mouthparts of a nymph of *I. hexagonus*.

4. STRUCTURE OF THE MOUTHPARTS *IN SITU*

The form and arrangement of the teeth on the hypostomes of all stages of *I. hexagonus* have been previously described (Arthur, 1951). Internally the hypostome (Fig. 2*a*) consists of two chambers,

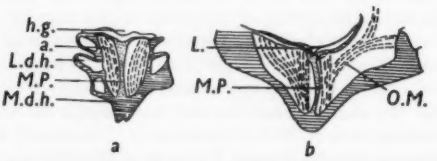


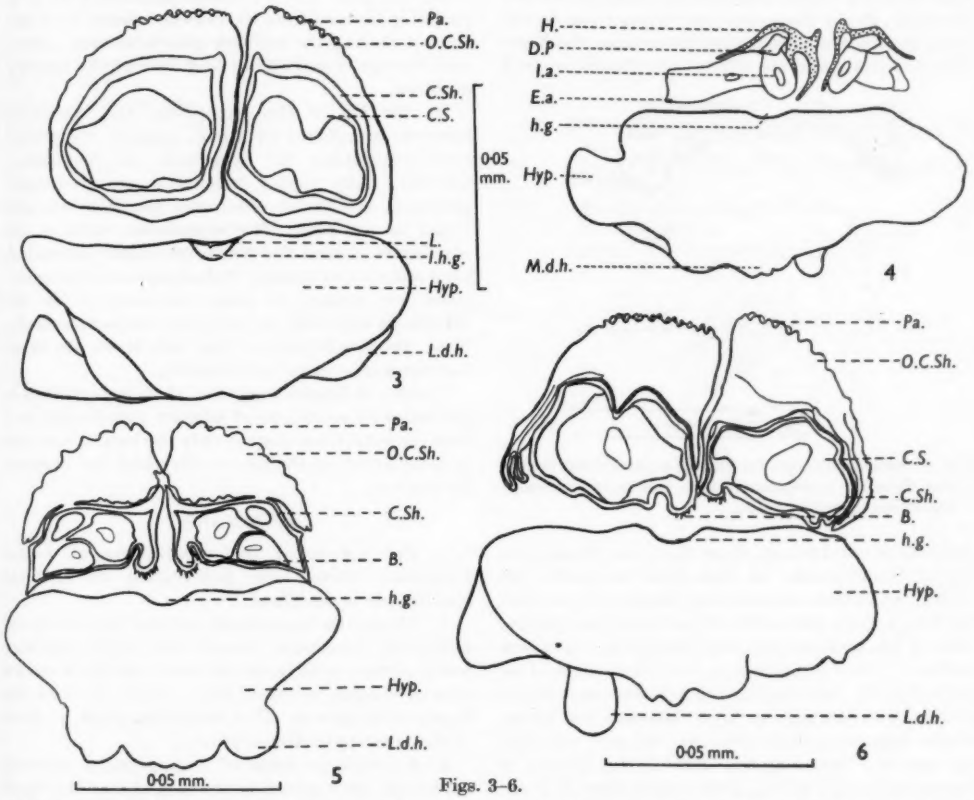
Fig. 2. Transverse sections through the hypostome of *I. hexagonus* at the level of the hypostomal groove (*a*), and at the base of the hypostome (*b*).

whose depth is greater than the breadth, and this composite structure suggests that it is derived from the fusion of a pair of lobes of the pedipalpal endites. Support for this is given in the description by Wagner (1894) of the development of the hypostome of *I. calcaratus* Berlese. The outer walls are hard and pigmented, whilst the separating median partition is paler and less rigid. Dorsally the hypostome is grooved along the midline to form the epithelium. For the greater part of its length the hypostome is provided with dorso-ventral muscles which, on contraction, will alter the form of the preoral canal, for it seems unlikely that they will affect the ventral and lateral walls because of their rigidity. More posteriorly, where hypostome and basis merge, the hypostomal muscles become oblique (Fig. 2*b*) and pass back to attachments on the dorso-lateral wall below the insertion of the palp. These I refer to as the hypostomal elevator muscles, for by their contraction they will raise the hypostome.

Above the labro-hypostomal groove and gutter, the chelicerae and their associated sheaths are cylindrical and complete (Fig. 3), but above the hypostomal groove their form and relationships are more complex. The digit, except for the pointed cusps and the cutting edges of the articles and dorsal process, is protected by a hood. The form of the latter cannot be determined from entire specimens because of its transparency, but it can be determined by means of serial sections. The hood (Fig. 4) lies on the dorsal and inner faces of the digit and reaches forward almost to the tip of the internal article. On the internal side the hood continues the outline of the cheliceral shaft forwards. The innermost sheaths at the apices of the chelicerae surround the latter completely (Fig. 5) and the inner

face of each of the sheaths is more or less flattened and closely apposed one against the other. The upper inner edges are produced upward while the lower inner edges, which bear downwardly directed teeth (Figs. 5, 6), are lobed. From my sections these toothed lobes appear to lie immediately above the hypostomal groove and extend back almost to the distal end of the labro-hypostomal groove. The outer and lower edges of the inner sheaths lie close against the upper surface of the hypostome (Fig. 5).

The outer cheliceral sheath is first seen at the apex of the shaft and appears as a triangularly shaped cushion lying dorso-laterally (Fig. 5). Strong papilla-like outgrowths are abundant on its dorsal surface, but they are less prevalent laterally. Farther back the toothed lobed processes are still evident, but the inner cheliceral sheaths are not completely free from the outer cheliceral sheaths. The latter still retain the thick cushion-like structures dorsally and from them folds hang down medially and laterally (Fig. 6). These folds ultimately coalesce to form the complete outer cheliceral sheaths, in front of the labro-hypostomal groove. Associated with the fusion there is a thinning of the dorsal cushions (Fig. 3).



Figs. 3–6.

Fig. 3. Transverse section through the mouthparts of *I. hexagonus* at the commencement of the labro-hypostomal groove.

Fig. 4. Transverse section through the digital portion of the capitulum of *I. hexagonus*.

Fig. 5. Transverse section through the apical region of the cheliceral shaft of *I. hexagonus*.

Fig. 6. Transverse section about midway along the hypostomal groove.

5. CHANGES IN THE HOST TISSUE FOLLOWING THE BITE

During feeding the rostrum penetrated into the corium and was surrounded by a more or less homogeneous zone, the cement of Cowdry & Danks. In both longitudinal and transverse sections the cement appears to be deposited in layers, but there

were no indications of finely granular layers alternating with the cement lamellae (cf. Cowdry & Danks, 1933). These lamellae are so arranged that they suggest that deposition occurs locally as the tick penetrates, and the net result is that the chelicerae and hypostome are ensheathed in this cement material. From the wound the cement has flowed along the lines of least resistance between the fibres of the dermis, and its effects are visible up to a

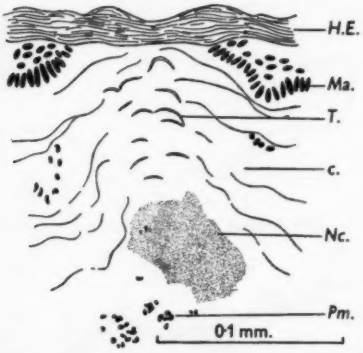


Fig. 7. Section through the skin of a parasitized hedgehog showing hypostomal teeth marks, the cement region and necrotic region.

distance of 0·5–1·0 mm., from the point of insertion of the mouthparts. In this zone no nuclei are visible, and where the secretion has flowed between the fibres, only the nuclei of the latter are visible. One of the sections was cut parallel to the lower surface of the hypostome and through some of its teeth (Fig. 7). Although no lesion was present in this section, the Malpighian layer beneath the hypostome was completely disorganized and occupied by cement. This suggests that if the cement is deposited locally during penetration then it is secreted at a very early stage and, in view of the widespread distribution of this substance, there can be little doubt that it was fluid in life.

Hoeppli & Feng (1931) and Cowdry & Danks (1933) have suggested that the 'cement' is a secretory product derived from the tick, and the latter authors have discussed the origin of the secretion. They put forward the following possibilities: (i) a secretion of the salivary glands, (ii) a secretion of the coxal glands, (iii) fluid regurgitated from the gut. The evidence suggests that it is probably derived from the salivary gland, for at the time of feeding the salivary glands are very active and contain eosinophilic and basophilic staining material.

At the tip of the hypostome the laminated material is replaced by a pale, opaque, coagulated mass indicating the condition of coagulation necrosis. Any nuclei remaining are frequently pyknotic, and small chromatin particles are also found in the mass. The endothelial walls of the capillaries around the lesion are much distended, producing haemorrhage. Polymorphonuclear leucocytes are present in some numbers within the capillaries and they are also seen extra-vascularly. Near the mouthparts of the tick there are small haemorrhagic areas in the dermis.

Cowdry & Danks suggested that the cytolysis is produced by secretions of salivary gland origin and that the cytolysins destroy only the local tissue cells without affecting the blood cells which are ingested by the tick.

SUMMARY

1. The action of the mouthparts of *Ixodes hexagonus* during the penetration of artificial membranes is described.

2. Above the hypostomal groove, the chelicerae are closely apposed in the mid-line, but on the inner lower surface each inner cheliceral sheath is drawn into a ventral, toothed lobe, which overlies the hypostomal groove. This lobe disappears in front of the labro-hypostomal groove.

3. A lamellate mass of homogeneous material surrounds the rostrum in the skin and at the tip of the hypostome there is marked cytolysis of the dermal cells. There are some pyknotic nuclei visible in this region, and the endothelial walls of the capillaries are swollen, and some contain many polymorphs.

REFERENCES

Arthur, D. R. (1951). The capitulum and feeding mechanism of *Ixodes hexagonus*, I. *Parasitology*, **41**, 66–81.

Cowdry, E. V. & Danks, W. B. C. (1933). Studies on East Coast Fever, II. Behaviour of the parasite and the development of distinctive lesions in susceptible animals. *Parasitology*, **25**, 1–63.

Hoeppli, R. & Feng, L. C. (1931). Histological reactions in the skin due to ecto-parasites. *Nat. Med. J. China*, **17**, 541–56.

Lees, A. D. (1948). The sensory physiology of the sheep tick, *Ixodes ricinus* L. *J. Exp. Biol.* **25**, 145–207.

Moskwin, J. A. (1929). Die Wirkung des Bisses der Zecke *Ornithodorus papillipes* Bir. auf die Haut der Versuchstiere. *Zbl. Bakt.* **110**, 208–13.

Pawlowsky, E. N. & Stein, A. K. (1927). Experimentelle Untersuchungen über die Wirkung von *Ixodes ricinus* (Ixodidae) auf die Menschenhaut. *Arch. Schiffs- u. Tropenhyg.* **31**, 574–86.

Wagner, J. (1894). Die Embryonalentwicklung von *Ixodes calcaratus* Bir. *Trav. Soc. Nat. St-Pétersb.* (*Leningr.*), **24**, 1–246.

Key to lettering of Text-figs. 1–7

a.	dorso-ventral muscles.
B.	median toothed lobes derived from the inner cheliceral sheath.
c.	'cement' layer.
C.S.	cheliceral shaft.
C.Sh.	inner cheliceral sheath.
C.Z.	tissue affected by tick secretion.
Di.	digit.
D.P.	dorsal process.
E.a.	external article of digit.
H.	hood of digit.
H.D.	dermis of host skin.
H.E.	horny layer of epidermis.
Hyp.	hypostome.
h.g.	hypostomal groove.
I.a.	internal article of digit.
L.	labrum.
L.d.h.	lateral denticles of hypostome.
l.h.g.	labro-hypostomal groove.
M.P.	median partition separating the two halves of the hypostome.
M.d.h.	median denticles of hypostome.
Ma.	Malpighian layer.
Nc.	necrotic region.
O.C.Sh.	outer cheliceral sheath.
O.M.	elevator muscles of hypostome.
P.	palp.
Pa.	papilla-like outgrowths on the outer cheliceral sheath.
Pm.	polymorphonuclear leucocytes.
S.g.	sebaceous gland.
T.	marks of hypostomal teeth.

(*MS. received for publication* 11. I. 52.—Ed.)

ON *ORNITHODOROS GRAINGERI* N.SP., A TICK FROM CAVES IN KENYA

By R. B. HEISCH, M.D. (Cantab.) and C. A. W. GUGGISBERG, *Division of Insect-Borne Diseases, Medical Research Laboratory, Kenya*

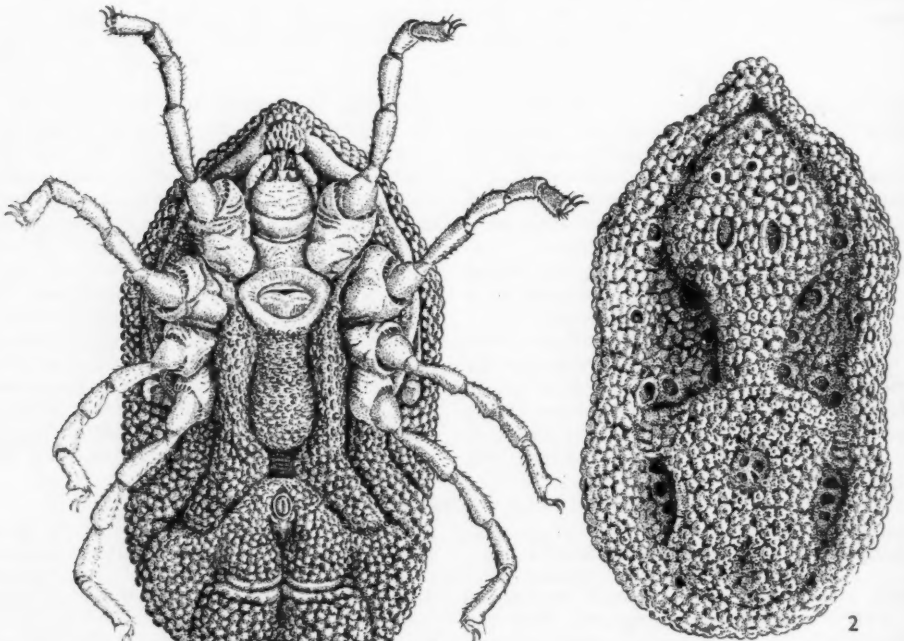
(With 19 Figures in the Text)

In April 1950, numerous ticks of the genus *Ornithodoros* were found in two coral caves, several hundred yards from the sea, near Diani about 20 miles south of Mombasa. Most of the ticks were found in the larger cave, which was irregular in shape, about 42 ft. long, and from 2 to 7 ft. high. Some of the ticks were crawling on the fine brown earth which covered the floors and others were just below the surface; none were seen on the walls.

Professor Theodor, when shown specimens, was of the opinion that they were a new species quite unlike other known African ticks of the same genus.

Ornithodoros graingeri n.sp. (Figs. 1–19.)

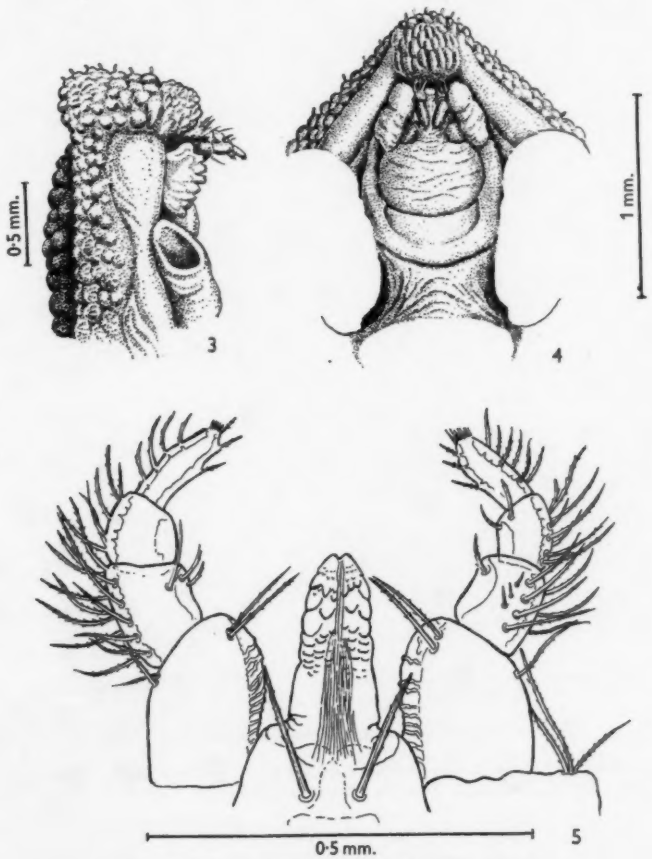
Female. Colour greyish-brown in live specimens. Size varying from 4 × 2 to 6 × 4 mm. No eyes. Body with sides almost parallel, but bulging slightly opposite coxae II and with a slight constriction level with coxae III and IV; anterior extremity narrowing to a round point; bluntly rounded posteriorly. Integument with numerous mammillae, some with short, spatulate hairs 50 μ in length; mammillae on dorsum stellate in appearance, particularly in distended specimens (see Fig. 2). Dorsal discs present (for



Figs. 1, 2. *Ornithodoros graingeri* n.sp.

Fig. 1. Female, ventral aspect. Fig. 2. Female, dorsal aspect.

arrangement see Fig. 2). *Venter*. Hood rather prominent and continuous with dorsum. Camerostome with lateral 'cheeks' and with 12–15 fine hairs on the anterior rim; posterior part usually a pronounced fold, occasionally indefinite; 'cheeks' of article I massive and stumpy with few hairs; article IV with a bunch of very short hairs on apex; articles I–IV measure respectively 180, 130, 90, 110 μ. Chelicerae with digit and bidentate process as shown in Fig. 6. Genital orifice (Fig. 7) opposite



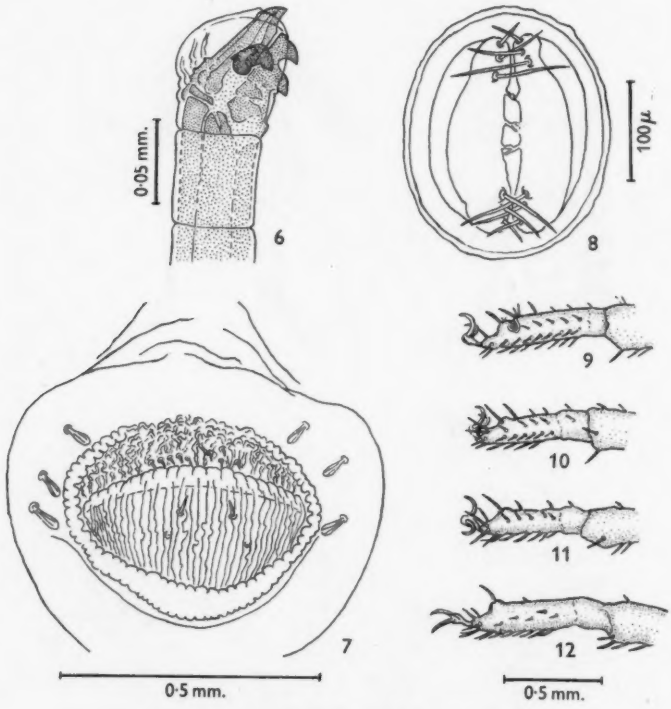
Figs. 3–5. *Ornithodoros graingeri* n.sp.

Fig. 3. Female, external surface of 'cheek' of camerostome. Fig. 4. Female, anterior portion of venter. Fig. 5. Female, hypostome and palps.

medium size and placed antero-laterally, their outer surfaces grooved and edges serrated (Figs. 3, 4). Basis capituli broader than long. Hypostome short (190 μ) and notched, with several rows of fine denticles distally, followed by stout teeth 2/2; number of teeth increasing to 3/3 and 4/4 basally, but decreasing in size; two short slightly serrated post-hypostomal hairs. Palps (Fig. 5) with numerous, long, stoutish hairs on articles II–IV; the interval between coxae I and II, separated from the posterior border of the camerostome by a raised rectangular area, which has slightly concave sides and triangular depressions on its posterior corners; its margin is raised and elliptically shaped. Anus (Fig. 8) oval (180 × 135 μ), about midway between posterior margin of body and coxae III; its valves with six pairs of hairs arranged in two groups at each end, increasing in size towards middle. Preanal

groove indefinite laterally, but its central part a well-defined rectangular depression with transverse striations. Transverse postanal groove clearly defined, divided into two halves by a median groove, which bisects it midway between the posterior

Spiracles crescentic (140 μ) and placed above, but in close contact with, the supracoxal folds, opposite coxae IV. Coxal folds parallel anteriorly, bulging inwards opposite the rectangular part of the preanal groove and extending outwards and backwards to



Figs. 6–12. *Ornithodoros graingeri* n.sp.

Fig. 6. Female, chelicera. **Fig. 7.** Female, genital orifice. **Fig. 8.** Female, anus. **Figs. 9–12.** Female (camera lucida drawings): **Fig. 9.** Tarsus I. **Fig. 10.** Tarsus II. **Fig. 11.** Tarsus III. **Fig. 12.** Tarsus IV.

border of the body and the anterior margin of the anus. Median postanal groove rather broad behind the anus, narrowing sharply when it crosses the transverse groove and then widening into a mammillated depression which ends at the posterior border; two small oval pits obliquely placed behind the anus. Each half of the transverse postanal groove at first straight, narrow, and at right angles to the median groove, with conspicuously smooth, unmammillated, lip-shaped margins; its outer parts shallower and less well defined, turning obliquely backwards to end at the posterior part of the lateral border. Supracoxal folds long, but not prominent, bending outwards and backwards to reach the lateral margin some distance behind the spiracle.

end in line with coxae IV; the coxal folds narrow considerably anteriorly, forming processes which pass between coxae I and II. *Legs* slender, light yellow in live specimens and with fine spines; 2nd and 3rd pairs shorter than 1st and 4th. Coxae decreasing in size from I to IV; coxa I slightly separated from coxa II, the rest contiguous. Short, broad, and slightly serrated hairs on the anterior and posterior rims of coxal articulations. Tarsi (Figs. 9–12) smooth with subapical dorsal protuberances, slight on I to III, more marked and rounded on IV; no dorsal humps present. Length of tarsus I, 0·06 mm.; metatarsus, 0·48 mm. Length of tarsus IV, 0·68 mm.; metatarsus, 0·60 mm.

Male. Smaller in size, varying from 3 × 1·5 to

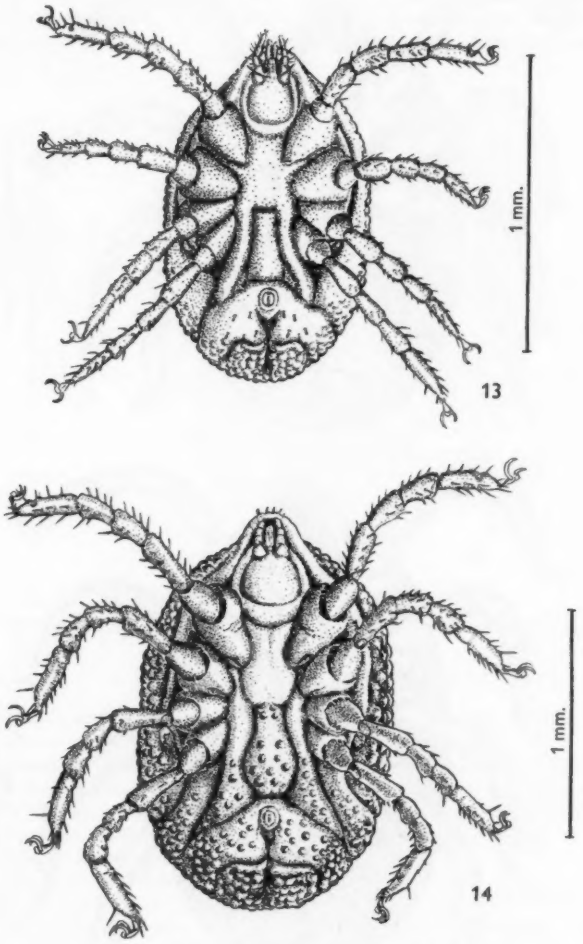
4·2 × 2·2 mm. Genital orifice more anterior than in the female, opposite coxae I; its margin insignificant with its anterior edge immediately behind the camerostome. Rectangular part of the preanal groove slightly different, being broader than long. Other characters similar to those of the female.

Nymphs. There are usually three and sometimes four nymphal stages and it is possible to separate one from the other. The different stages are now briefly described.

First stage (Fig. 13) from 1·2 to 1·5 mm. long. Ventral surface almost devoid of mammillae, but some mammillae present behind the transverse postanal groove and along the margins. Preanal groove simple and without rectangular depression.

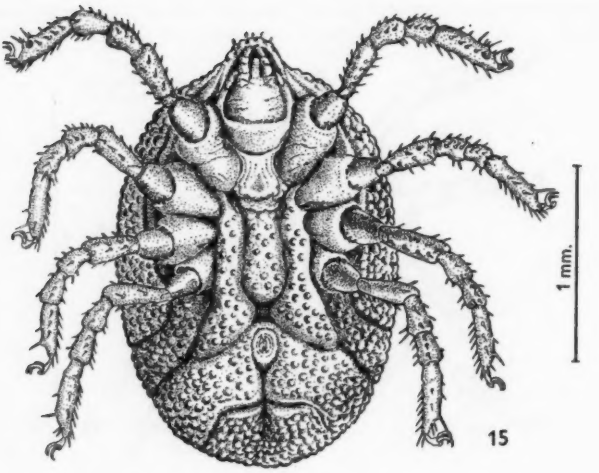
Second stage (Fig. 14) about 1·8 mm. long. Ventral mammillae like those of the first stage, but also sparsely scattered on and between the coxal folds. Preanal groove still simple.

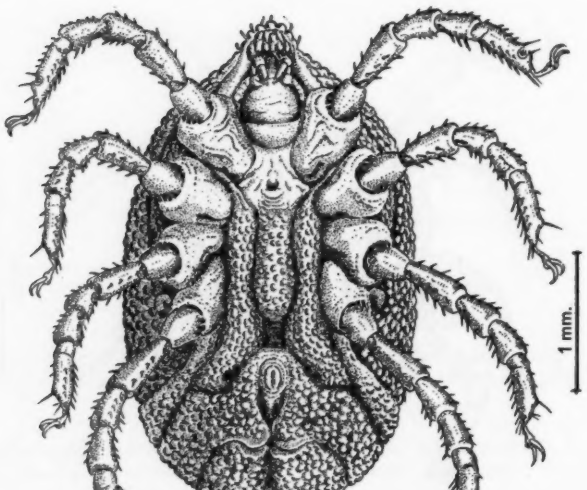
Third stage (Fig. 15) from 2 to 2·2 mm. long.



Figs. 13, 14. *Ornithodoros graingeri* n.sp.

Fig. 13. First stage nymph, ventral aspect. Fig. 14. Second stage nymph, ventral aspect.





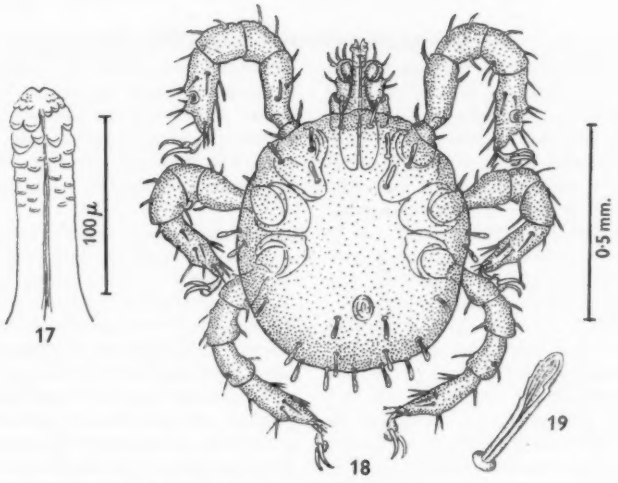
Figs. 15, 16. *Ornithodoros graingeri* **n.sp. Ventral aspects.**
Fig. 15. Third stage nymph. Fig. 16. Fourth stage nymph.

Ventral mammillae more numerous, but still fewer than in the adult. Rectangular depression of the preanal groove now apparent.

Fourth stage (Fig. 16) from 2·5 to 3·5 mm. long. Ventral mammillae as in the adult. A depression at the site of the future genital orifice. Hood more prominent than in the earlier stages.

about twice as long as the hypostome and provided dorsally with serrated hairs.

Described from holotype, allotype, and 36 paratypes (17 males, 14 females, 4 nymphs of different stages, 1 larva), and numerous other specimens. The holotype, allotype, and 36 paratypes have been presented to the British Museum. We have much



Figs. 17–19. *Ornithodoros graingeri* n.sp.

Fig. 17. Hypostome of second stage nymph. Fig. 18. Larva dorsal aspect.
Fig. 19. Dorsal hair.

Tarsal, subapical, dorsal protuberances are not present in the first three nymphal stages. The 'cheeks' of the camerostome begin to appear as small folds in the second nymphal stage and are well developed in the third stage. Suction pads are present on the tarsi of the first three stages, but are absent from adults. The anus of the first stage with only two pairs of short hairs. The hypostomes of the nymphal stages simpler than in the adult; that of second stage shown in Fig. 17.

Larva. Suboval to oval (Figs. 18, 19); length 600–810 μ without, and 690–940 μ with, hypostome. Dorsum with serrated club-shaped hairs, 63–70 μ in length; five to six of these project from the posterior margin. Other hairs as shown in Fig. 18. Venter with short, spiny hairs. Legs long and slender, the middle pair shorter than the others; tarsi with well-developed suction pads; coxae I and II slightly separated. The capitulum, although it is inserted ventrally, projects well in front of the body. Hypostome short and notched, denticles arranged 2/2; distal row small, next two rows coarse and blunt, remainder insignificant. Palps

pleasure in naming this tick after its collector Mr W. E. Grainger.

Host. Probably porcupines, many quills being present in the caves. Several bats (*Rhinolophus* and *Megaderma* sp.) were caught in one of the caves, but all were free of ticks.

DISCUSSION

The presence of 'cheeks' on the camerostome suggested that *Ornithodorus graingeri* might be related to the *talaje* group of ticks. A comparison was therefore made with *O. talaje*, *O. coniceps*, and *O. capensis*, specimens of which were kindly provided by Mr Browning of the British Museum and Dr Gordon E. Davis.

O. graingeri was found to be quite unlike these ticks, which have divergent coxal folds, simple preanal grooves without rectangular depressions, the tarsi devoid of subapical dorsal protuberances and the transverse postanal groove untraversed by the median postanal groove.

An unusual character which probably distin-

guishes the larvae of *O. graingeri* from those of other known African species of *Ornithodoros*, is the presence of club-shaped hairs on the dorsum and posterior margin; these hairs are usually longer and pointed in other species. The larvae of *O. turicata* have hairs which are slightly clubbed, the adult also resembling *O. graingeri* particularly in the arrangement of the coxal folds and anal grooves. The American tick, however, is larger and has different mammillae and tarsi and its camerostome is devoid of 'cheeks'.

We have to thank the Director of Medical Services, Kenya, for permission to publish this paper and Mrs E. C. C. van Someren for much helpful advice.

(*MS. received for publication* 3. IX. 1952.—Ed.)

THE MITES (ACARINA: ANALGESIDAE) LIVING ON OR IN THE FEATHERS OF BIRDS

By CHARLES D. RADFORD, *Membre Correspondant, Muséum National d'Histoire Naturelle, Paris*

In 1950 the author published a paper entitled 'The Mites (Acarina) parasitic on Mammals, Birds and Reptiles' in which it was stated that the subject of feather mites (Analgesidae) was too big to be included in that work, and though they were parasitic throughout their life upon the host or in the quills of the feathers they should be made the subject of a separate paper.

Most of these feather mites are to be found in the web of the wing or body feathers, though occasionally one finds them inhabiting the shafts of the feathers. Whether or not they are carriers of infection is not certain, but in view of the nature of their food it would appear to be unlikely. A study of their chelicerae, furnished as they are with teeth, points to the fact that they feed upon the quill tissue and not upon the blood or sebaceous fluids. Judging from the large numbers of these mites, often of a single species, which one finds upon a bird, it seems most probable that their presence is not annoying to the host. Where the quill-inhabiting species are present, it is invariably found that they are not in the tip of the quill below the skin of the host, but are some distance above the skin. In life, the species which live in the web of the feathers are very active when disturbed, but spend a large part of their life feeding steadily with little movement. Where more than one species is found upon the same host they are not unusually found feeding in close proximity with one another. Cross-breeding is not recorded. Some species are host specific. Others are to be found on birds of the same family, as for example *Gabucinia delibata* which is found on many species of the family Corvidae. *Kramerella lunula*, however, is found only on the love birds—budgerigars; and *Dermoglyphus* only on the pigeon.

The bulk of our knowledge of these feather mites is due to the work of Canestrini, Trouessart, Megnin and Neumann and was most ably summarized by Canestrini & Kramer (1899) in Lieferung 7 of *Das Tierreich*. Since then little has been done on this group of mites with the exception of the papers by Trouessart, and more recently the papers by Bonnett. The various papers in which the original descriptions appeared are now not readily accessible, having been published in several languages and in scattered scientific journals, and the present paper should fill a gap in bringing the work of all these authors into one source.

Family ANALGESIDAE

Subfamily Pterolicheae Megnin, 1884

Genus **Freyana** Haller, 1877

F. anatina C. L. Koch, 1844	*Anas acuta acuta* L. 1758
	A. crecca crecca L. 1758
	A. querquedula L. 1758
	Mergus serrator L. 1758
	Aythya fuligula (L. 1758)
	Nettapus auritus (Boddaert, 1783)
F. anserina Trouessart & Megnin, 1884	*Anser anser anser* (L. 1758)
	A. hyperboreus hyperboreus (Pallas, 1769)
	Cygnus olor (Gmelin, 1789)
F. chorioptoides Trouessart & Megnin, 1884	*Theristicus carunculatus* (Ruppel, 1835)
F. gracilipes Trouessart & Megnin, 1884	*Grus antigone antigone* (L. 1758)
	Ephippiorhynchus senegalensis (Shaw, 1798)
F. grandiloba Trouessart & Neumann, 1888	*Rhynchops nigra nigra* L. 1758
F. halleri Trouessart & Megnin, 1884	*Platalea ajaja* L. 1766
F. horrida Trouessart & Megnin, 1884	—
F. leclerci Trouessart, 1886	*Afribyx senegalus senegalus* (L. 1766)
	Lobivanellus indicus indicus (Boddaert, 1783)

F. marginata Trouessart, 1886	*Rhynchops flavirostris* Vieillot, 1816
F. oblongo Trouessart & Neumann, 1888	*Theristicus leucocephalus* Reich, 1894
F. pectinata Trouessart, 1886	*Scopus umbrettus* Gmelin, 1788
F. pelargica Trouessart & Megnin, 1884	*Ciconia ciconia ciconia* (L. 1758)
	C. nigra (L. 1758)
	Euxenura maguari (Gmelin, 1788)
F. tarandus Trouessart & Neumann, 1888	*Ibis melanocephala* (Latham, 1787)
	I. molucca Cuvier, 1829
	Theristicus melanopis (Gmelin, 1788)

Genus **Microchelys** Trouessart, 1915

M. delicatula (Trouessart, 1898)	*Macropteryx mystacea* (Lesson, 1826)

Subgenus **Halleria** Trouessart & Megnin, 1885

H. ceratorhina Trouessart, 1886	*Tantalus ibis* L. 1766
H. hirsutirostris Trouessart & Megnin, 1885	*Phoenicopterus ruber roseus* Pallas, 1811

Subgenus **Michaelichus** Trouessart & Megnin, 1885

Syn. *Michaelia* Trouessart, 1884 sed non Michaelea Berlese, 1884	
M. caput-medusae Trouessart, 1886	*Sula bassana* (L. 1758)
M. heteropus (Michael, 1881)	*Bugeranus aristotelis aristotelis* (L. 1761)

Subgenus **Microspalax** Megnin & Trouessart, 1884

M. brevipes Megnin & Trouessart, 1884	*Puffinus assimilis baroli* (Bonaparte, 1857)
M. chanayi Trouessart, 1885	*Meleagris gallopavo* L. 1758
M. delicatula Trouessart, 1898	*Macropteryx mystacea* (Lesson, 1826)
M. gigas Trouessart, 1895	*Diomedea nigripes* Audubon, 1839
M. major Trouessart & Neumann, 1888	*Daption capense* (L. 1758)
M. manicata Megnin & Trouessart, 1884	*Puffinus* sp.

Subgenus **Kramerella** Trouessart, 1915

Syn. *Crameria* Haller, 1878 pre-occ.	
K. lunulata (Haller, 1878)	Strigidae
K. lyra (Canestrini, 1878)	Strigidae
K. major (Haller, 1878)	Strigidae

Genus **Pterolichus** Robin, 1868

P. anomalus Trouessart & Neumann, 1888	*Theristicus carunculatus* (Ruppell, 1835)
P. ardeae (Canestrini, 1878)	*Botaurus stellaris stellaris* (L. 1758)
P. attenuatus Megnin & Trouessart, 1884	Bucerotidae
P. bicaudatus (Gervais, 1844)	*Struthio camelus* L. 1766
	Rhea americana (L. 1758)
P. biemarginatus Megnin & Trouessart, 1884	*Capito niger auratus* (Dumont, 1816)
	Trogon collaris Vieillot, 1817
P. canestrinii Trouessart, 1885	*Ara macao* (L. 1758)
	A. canga Levaill, 1801
	A. severa severa (L. 1758)
P. cataphractus Megnin & Trouessart, 1884	*Tragopan satyra* (L. 1758)
	Calornis chalybea (Horsfield, 1820)
P. charadrii (Canestrini, 1878)	*Charadrius hiaticula* L. 1758
	C. a. alexandrinus L. 1758
	C. dubius curonicus Gmelin, 1789
	Actitis hypoleucos (L. 1758)
P. ciconiae Canestrini & Berlese, 1880	*Ciconia c. ciconia* (L. 1758)
P. circiniger Megnin & Trouessart, 1884	*Rhytidoceros plicatus* (Forsten, 1781)
	Cranorrhinus leucocephalus (Vieillot, 1816)
P. coccyger Trouessart & Neumann, 1888	*Aramus s. scolopaceus* (Gmelin, 1789)

P. columbae Sugimoto, 1940	Pigeon
P. corystes Trouessart & Neumann, 1888	*Bugeranus carunculatus* (Gmelin, 1789)
P. crassus Megnin & Trouessart, 1884	*Anthracoceros coronatus* (Boddaert, 1783)
P. cribriformis Megnin & Trouessart, 1884	*Forpus p. passerinus* L. 1758
P. cuculi Megnin & Trouessart, 1884	*Cuculus canorus canorus* L. 1758
	Merops apiaster L. 1758
P. decoratus Megnin & Trouessart, 1884	*Rollulus roulroul* (Scopoli, 1786)
P. denticulatus Megnin & Trouessart, 1884	*Pyrrhura cruentata* Wied, 1820
P. elegans (Trouessart, 1897)	*Cyclopsitta* sp.
P. eventratus Megnin & Trouessart, 1884	*Carine passerina* (L. 1758)
P. freyanoides Trouessart, 1898	*Corvus senex* Lesson, 1828
P. gracilis Trouessart, 1886	*Megapodius reinwardt forsteni* Gray, 1847
	M. f. freycineti Gaimard, 1823
P. grandis Trouessart & Neumann, 1888	*Childonias n. nigra* (L. 1758)
P. hastifolia Megnin & Trouessart, 1885	*Pyrrhura ferruginea* (Muller, 1776)
P. hirundo Megnin & Trouessart, 1884	*Harpyia destructor* Cuvier, 1817
P. hystrix Trouessart, 1898	*Opisthocomus hoaxin* (Muller, 1776)
P. inermis Megnin & Trouessart, 1884	*Caica leucogastra* (Kuhl, 1820)
P. longiventer Megnin & Trouessart, 1884	*Strix nebulosum* (Forsten, 1772)
P. marginatus Trouessart, 1886	*Theristicus leucocephalus* Reich, 1894
P. marinus Trouessart, 1886	*Larus argentatus* Pontoppidan, 1763
P. martini Trouessart, 1885	*Sterna h. hirundo* L. 1758
P. microphyllus Megnin & Trouessart, 1884	*Caica melanocephala* (L. 1766)
P. m. var. *porrecta* Megnin & Trouessart, 1884	*Pionus menstruus* (L. 1766)
P. minor Megnin & Trouessart, 1884	*Falco s. subbuteo* L. 1758
P. musophagi Trouessart, 1886	*Schizoris africana* (Latham, 1789)
	Turacus buffoni (Vieillot, 1819)
P. ninii (Canestrini, 1878)	*Numenius a. arquata* (L. 1758)
	N. tenuirostris Vieillot, 1817
P. obtusus Robin, 1877	*Perdix rufa* (L. 1766)
	Perdix saxatilis (Meyer, 1805)
P. onychophorus Trouessart, 1898	*Brachypteracias crossleyi* (Sharpe, 1877)
P. o. var. *fauna* Trouessart, 1898	*B. pittoides* Lafresnaye, 1834
	B. crossleyi Sharpe, 1877
P. ornatus Megnin & Trouessart, 1884	—
P. parallelilobus Trouessart, 1886	*Otus scops scops* (L. 1758)
P. parallelus Megnin & Trouessart, 1884	*Turacus buffoni* (Vieillot, 1819)
P. parrae Megnin & Trouessart, 1884	*Hydrophasianus chirurgus* (Scopoli, 1786)
P. phylloproctus Megnin & Trouessart, 1884	*Haliaetus leucogaster* (Gmelin, 1788)
P. p. var. *minor* Megnin & Trouessart, 1884	*Haliastur indus girrenera* (Vieillot, 1822)
P. phyllurus Trouessart, 1898	*Otidiphaps nobilis* Gould, 1870
P. p. var. *ulocerca* Trouessart, 1898	*Probosciger a. aterrimus* (Gmelin, 1788)
P. pustulatus Megnin & Trouessart, 1884	*Crax alector* L. 1766
P. pyroderi Megnin & Trouessart, 1884	*Pyroderus scutatus* (Shaw, 1792)
P. rhamphastinus Megnin & Trouessart, 1884	*Rhamphastos discolorus* L. 1766
P. rubidus Trouessart, 1886	*Diomedea chlororhyncha* (Gmelin, 1788)
P. r. var. *petalifera* Trouessart, 1898	*D. chlororhnycha* (Gmelin, 1788)
P. serrativentris Trouessart, 1886	*Leptoptilus argala* Gray, 1844
P. umbellifer Trouessart, 1898	*Opisthocomus hoazin* (Muller, 1776)
P. uncinatus Megnin, 1887	*Steganura paradisea* (L. 1766)
P. varians Trouessart, 1898	*Micropus melba* (L. 1758)
P. v. var. *selenura* Trouessart, 1898	*Chaetura zonaris* (Shaw, 1796)
P. venustissimus Trouessart, 1898	*Conurus c. canicularis* (L. 1758)
P. xiphiurus Megnin & Trouessart, 1898	*Charadrius dubius curonicus* Gmelin, 1789

Genus **Ceratothrix** Trouessart, 1915

C. corniger Trouessart & Neumann, 1888	*Psophia c. crepitans* L. 1758

Genus **Avenzoaria** Oudemans, 1905

A. limosae (Buchholz, 1869)	Limosae
A. l. var. *mucronata* (Megnin & Trouessart, 1884)	*Porzana f. fusca* (L. 1766)
A. l. var. *selenura* (Megnin & Trouessart, 1884)	*Catoptrophorus s. semipalmatus* (Gmelin, 1789)
A. numenii (Canestrini, 1878)	*Numenius phaeopus* (L. 1758)
A. ochropodus Hull, 1931	*Tringa ochropus* L. 1758
A. squatarolae (Canestrini, 1878)	*Squatarola squatarola* (L. 1758)
A. totani (Canestrini, 1878)	*Tringa totanus* (L. 1758)
	Philomachus pugnax (L. 1758)
	Erolia a. alpina (L. 1758)

Genus **Ptiloxenus** Hull, 1934

P. buchholzi (Canestrini, 1878)	*Limosa l. limosa* (L. 1758)
	Squatarola squatarola (L. 1758)
P. b. var. *hastigera* (Megnin & Trouessart, 1884)	*Erolia a. alpina* (L. 1758)
	Microsarcops cinereus (Blyth, 1842)
P. b. var. *fascigera* (Megnin & Trouessart, 1884)	*Tringa totanus* (L. 1758)
	Calidris canutus canutus (L. 1758)
	Arenaria interpres (L. 1758)
P. b. var. *securicata* (Megnin & Trouessart, 1884)	*Erolia testaceae* (Pallas, 1764)
P. colymbi (Canestrini, 1878)	*Colymbus fluviatilis* Tunst. 1771
P. major (Megnin & Trouessart, 1884)	*Podiceps c. cristatus* (L. 1758)
	Urinator septentrionalis (L. 1766)
P. microphyllus (Megnin & Trouessart, 1884)	*Turacus meriani* (Ruppell, 1851)
P. phoenicopteri (Megnin & Trouessart, 1884)	*Phoenicopterus ruber roseus* Pallas, 1811
P. rehbergi (Canestrini & Berlese, 1880)	*Haematopus ostralegus* L. 1758
P. r. var. *gracilis* (Megnin & Trouessart, 1884)	*Himantopus h. himantopus* (L. 1758)
P. vanelli (Canestrini, 1878)	*Vanellus vanellus* (L. 1758)
P. vexillarius (Megnin & Trouessart, 1884)	Bucerotidae
P. v. var. *homophylla* (Megnin & Trouessart, 1884)	Bucerotidae
P. v. var. *minor* (Megnin & Trouessart, 1884)	*Hydrocorax hydrocorax* (L. 1766)
P. v. var. *minuta* (Megnin & Trouessart, 1884)	*Lophoceros melanoleucus* (Lichtenstein, 1793)
	L. erythrorhynchus (Temminck, 1823)

Genus **Grallobia** Hull, 1934

G. fulicae (Trouessart, 1885)	*Fulica atra atra* L. 1758
G. porzanae (Canestrini, 1878)	*Porzana porzana* (L. 1766)
G. proctogamae (Trouessart, 1885)	*Fulica atra atra* L. 1758
	Porphyrio caeruleus (Vandelli, 1797)
	Hypotaenidia philippensis (L. 1766)
G. rallorum (Robin, 1877)	*Crex crex* (L. 1758)

Genus **Cynthiura** Hull, 1934

C. aquilinus (Trouessart, 1885)	*Aquila chrysaetus* (L. 1758)
	A. pomarina Brehm 1831
	Haliaetus sp.
C. a. var. *milvulina* (Trouessart, 1884)	*Milvus m. milvus* (L. 1758)
	Haliastur indus girrenera (Vieillot, 1822)
C. microdiscus (Trouessart, 1886)	*Tetrao u. urogallus* L. 1758

Genus **Hirstia** Hull, 1931

H. chelidonis Hull, 1931	*Martula u. urbica* (L. 1758)

Genus **Brephosceles** Hull, 1934

B. forficiger (Megnin & Trouessart, 1884)	*Urinator glacialis* (L. 1766)
B. ortygometrae (Canestrini, 1878)	*Porzana pusilla* (Pallas, 1776)
	Porzana porzana (L. 1766)
B. o. var. *furcifer* (Trouessart, 1885)	*Cursorius bicinctus* Temminck, 1820

Genus **Gabucinia** Oudemans, 1905

G. delibata (Robin, 1877)	*Corvus corone corone* L. 1758
	C. cornix cornix L. 1758
	C. corax corax L. 1758
	C. frugilegus frugilegus L. 1758
	C. scapulatus Daudin, 1800
G. intermedia (Megnin & Trouessart, 1884)	Birds of prey
G. nisi (Canestrini, 1878)	*Accipiter n. nisus* (L. 1758)
	Circaetus gallicus (Gmelin, 1788)
	Buteo b. buteo (L. 1758)
	Pernis a. apivorus (L. 1758)
	Circus pygargus (L. 1758)
G. otidis (Megnin & Trouessart, 1884)	*Chlamydotis u. undulata* (Jacquin, 1784)
G. pallida (Megnin & Trouessart, 1884)	*Burhinus o. oedicnemus* (L. 1758)
G. urogalli (Norner, 1883)	*Tetrao u. urogallus* L. 1748

Genus **Eustathia** Oudemans, 1905

E. cultrifera (Robin, 1877)	*Micropus a. apus* (L. 1758)
	M. melba (L. 1758)

Genus **Chauliacia** Oudemans, 1905

C. securiger (Robin, 1877)	*Micropus a. apus* (L. 1758)

Genus **Anoplonotus** Trouessart, 1915

A. semaphorus (Trouessart, 1886)	*Sterna h. hirundo* L. 1758

Genus **Pseudalloptes** Trouessart & Megnin, 1884

P. berlesei Megnin & Trouessart, 1884	*Rhytidoceros plicatus* (Forsten, 1781)
P. bimucronatus Trouessart, 1884	Lagopidae
	Phasianidae
P. bisubulatus (Robin, 1877)	*Perdix p. perdix* (L. 1758)
	P. rufa (L. 1758)
P. calcaratus Trouessart, 1884	Bucerotidae
P. cultriventris Trouessart, 1884	*Glossopsitta concinna* (Shaw, 1791)
P. curtus Trouessart, 1886	*Megapodius f. freycineti* Gaimard, 1823
	Aepypodius bruijni (Oustalet, 1880)
P. delibativentris Trouessart, 1884	*Domicella domicella* (L. 1758)
P. discifer Trouessart, 1884	*D. domicella* (L. 1758)
P. emargiventris Trouessart, 1884	*Coryphilus taitianus* (Gmelin, 1788)
P. fissiventris Trouessart, 1885	Bucerotidae
	Penelopides manillae (Boddaert, 1783)
	Rhytidoceros plicatus (Forster, 1781)
	Anthracocerus malabaricus (Gmelin, 1788)
P. forficiventris Trouessart, 1884	*Caica leucogastra* Kuhl, 1820
P. forficula Trouessart & Neumann, 1888	*Ortalis araucuan squamata* Lesson, 1829
P. furstenbergi (Buchholz, 1869)	Bucerotidae
	Buceros rhinoceros L. 1766
	Lophoceros leucolophus (Sharpe, 1873)
	Anthracoceros malabaricus (Gmelin, 1788)
	Hydrocorax hydrocorax (L. 1766)
P. gruis Trouessart, 1884	*Grus grus grus* (L. 1758)
P. lambda Trouessart, 1899	*Coracopsis vasa comorensis* (Peters, 1854)
P. lobiger Trouessart, 1884	*Domicella domicella* (L. 1758)
P. machetes Trouessart, 1898	*Poicephalus gulielmi* (Jardine, 1849)
P. ogivalis Megnin & Trouessart	*Rhytidoceros plicatus* (Forsten, 1781)
P. palmiger Trouessart, 1886	*Talegalla cuvieri* Lesson, 1828
	Aepypodius bruijni (Oustalet, 1880)

P. panoplites Trouessart, 1898	*Poicephalus gulielmi* (Jardine, 1849)
P. proctophyllus Trouessart, 1898	*Colius nigricollis* Vieillot, 1817
P. pterocolurus Trouessart, 1885	Bucerotidae
	Anthracoceros convexus (Temminck, 1832)
	Penelopides manillae (Boddaert, 1783)
P. pyriventris Trouessart, 1886	*Scopus umbretta* Gmelin, 1788
P. p. var. *vegetans* Trouessart, 1899	*S. umbretta* Gmelin, 1788
P. quadratus Trouessart, 1886	*Aepypodius bruijni* (Oustalet, 1880)
	Talegallus cuvieri Lesson, 1828
P. securiventris Trouessart, 1884	*Coryphilus taitianus* (Gmelin, 1788)
P. spathuliger Trouessart, 1884	*Calyptorhynchus magnificus macrorhynchus* Gould, 1842
P. tenuis Trouessart, 1884	*Pionus menstruus* (L. 1766)
P. thoracosathes Trouessart & Neumann, 1888	*Megapodius f. freycineti* Gaimard, 1823
P. tridentiger Trouessart, 1886	*M. reinwardt forsteni* Gray, 1847
P. tritilobus Trouessart, 1886	*M. r. forsteni* Gray, 1847
P. tritiventris Trouessart, 1884	*Ara* sp.
	Conurus sp.
P. t. var. *ulocerea* Trouessart, 1898	*Ara cloroptera* Gray, 1859
P. t. var. *dilatata* Trouessart, 1898	*Ara n. nobilis* (L. 1758)

Genus **Oustaletia** Trouessart, 1885

O. pegasus Trouessart, 1885	Bucerotidae
	Anorrhinus galeritus (Temminck, 1824)
	Rhytidoceros plicatus (Forsten, 1781)
	Hydrocorax hydrocorax (L. 1758)
O. p. var. *retusa* Trouessart, 1885	*Anthracoceros malabaricus* (Gmelin, 1788)
	Anorrhinus galeritus (Temminck, 1824)

Genus **Cheylabis** Trouessart, 1885

C. latus Trouessart, 1885	—
C. praecox Trouessart, 1885	—

Genus **Columellaia** Oudemans, 1904

C. varians (Trouessart, 1886)	*Numida m. meleagris* (L. 1758)

Genus **Paralges** Trouessart, 1885

P. pachycnemis Trouessart, 1885	*Struthio camelus* L. 1766
	Rhea americana (L. 1758)

Genus **Protonyssus** Trouessart, 1915

P. larva (Trouessart, 1885)	Psittacidae

Genus **Bonetella** Trouessart, 1924

Syn. *Buchholzia* Trouessart, 1915 pre-occ.	
B. fuscus (Nitzsch, 1818)	*Pandion h. haliaetus* (L. 1758)

Genus **Pseudogiebelia** Radford, 1950

Syn. *Giebelia* Trouessart, 1915 pre-occ.	
P. puffini (Buchholz, 1869)	*Dromas ardeola* Paykull, 1805
	Sternidae
	Laridae
	Puffinidae
	Procellariae

Genus **Pteronyssus** Robin, 1868

P. acris Trouessart & Neumann, 1888	*Megapodius l. laperouse* Gaimard, 1823
P. bifidus Trouessart, 1885	*Capito n. niger* (Muller, 1776)

P. brevipes Berlese, 1884	*Dendrocopus medius* (L. 1758)
P. chiasma Trouessart, 1885	*Pteroglossus a. aracari* (L. 1758)
P. c. var. *mucronata* Trouessart, 1885	*Rhamphastos dicolorus* L. 1766
P. conurus Trouessart, 1886	*Lybius b. bidentatus* (Shaw, 1798)
	L. bilineatus leucolaima (Verreaux, 1861)
P. c. var. *dislocata* Trouessart, 1886	*L. b. bidentatus* (Shaw, 1798)
P. gracilis (Nitzsch, 1818)	Picidae
P. hipposathes Trouessart, 1886	*Astrapia nigra* (Gmelin, 1788)
P. integer Trouessart & Neumann, 1888	*Muscicapa striata* (Pallas, 1764)
P. latior Trouessart, 1886	*Astrapia nigra* (Gmelin, 1788)
P. phyllophorus Trouessart, 1885	*Musophaga violacea* Isert, 1789
P. simplex Haller, 1882	*Melanerpes e. erythrocephalus* (L. 1758)
P. spathuliger Trouessart, 1885	*Celeus e. elegans* (Muller, 1776)
P. speciosus Tyrrell, 1882	*Dendrocopus villosus* (L. 1766)
P. tyrrelli Canestrini, 1899	*Tachycineta bicolor* (Vieillot, 1807)
Syn. *P. fuscus* Tyrrell, 1882	

Genus **Pteronyssoides** Hull, 1931

P. nuntiaeveris (Berlese, 1884)	*Riparia r. riparia* (L. 1758)
P. obscurus (Berlese, 1884)	*Martula u. urbica* (L. 1758)
	Riparia r. riparia (L. 1758)
P. pallens (Berlese, 1884)	*Acrocephalus a. arundinaceus* (L. 1758)
P. parinus (Koch, 1840)	*Parus caeruleus* L. 1760
P. quadratus (Haller, 1882)	*Picus* sp.
	Sylvia a. atricapilla (L. 1758)
P. striatus (Robin, 1877)	*Fringilla c. coelebs* L. 1758
P. truncatus (Trouessart, 1885)	*Sturnus v. vulgaris* L. 1758
	Lamprotornis sp.
P. t. var. *subtruncatus* (Trouessart, 1885)	*Mainatus javanensis* (Horsfield, 1820)
	Calornis panayensis (Scopoli, 1783)

Genus **Xoloptes** Canestrini, 1879

X. claudicans (Robin, 1877)	*Coturnix c. coturnix* (L. 1758)
	Perdix p. perdix (L. 1758)
	P. rufa (L. 1766)
X. didactylus Trouessart, 1885	*Ciconia c. ciconia* (L. 1758)
X. minor Trouessart & Neumann, 1888	*Ortalis araucuan squamata* Lesson, 1829

Family ANALGESIDAE Trouessart, 1915

Genus **Mesalges** Trouessart, 1888

M. abbreviatus (Buchholz, 1869)	*Buceros rhinoceros* L. 1766
	Corvus cornix cornix L. 1758
	Rhinoplax vigil (Forsten, 1781)
M. bicalcaratus (Trouessart, 1886)	*Francolinus levaillanti* (Valenciennes, 1825)
M. ceratopus Bonnet, 1924	*Melanorhectes nigrescens* (Schlegel, 1871)
	Oriolus striatus Quoy & Gaimard, 1830
	Rhectes cirrhocephelus Lesson & Garnott, 1827
	Pinarolestes heinei (Finoch & Hartert, 1869)
M. characurus Trouessart, 1899	*Psittrichus pecquetii* (Lesson, 1831)
M. circiniger Trouessart, 1886	*Sula fiber* (L. 1766)
M. diaphanoxus Bonnet, 1924	*Corvus scapulatus* Daudin, 1800
	Macrocorax fuscicapillus (Gray, 1859)
	Corone orru (Bonaparte, 1851)
	Melilestes megarhynchus Gray, 1858
	Heteralocha gouldi Cabanis, 1850
	Ocydromus earlei Gray, 1862
	Glaucopis cinerea Gmelin, 1788

M. elephantopus Trouessart & Neumann, 1888	*Rhytidoceros plicatus* (Forsten, 1781)
M. hepatifolia Bonnet, 1924	*Rhinoplax vigil* (Forsten, 1781)
M. hirsutus Trouessart, 1899	*Aratinga solstitialis* (L. 1758)
	Conurus leucotis Kuhl, 1820
	C. c. canicularis (L. 1758)
	Nandayus nenday (Vieillot, 1823)
	Caica melanocephala (L. 1766)
	Pyrrhura picta picta (Muller, 1776)
	P. l. leucotis (Kuhl, 1820)
	Nasiterna pygmae (Quoy & Gaimard, 1830)
	Furnarius torridus Sclater & Salvin, 1866
	Megarhynchus pitangua (L. 1766)
	Cirrhopipra filicauda (Spix, 1825)
	Brotogeris versicolurus chiriri (Vieillot, 1817)
	Hartlaubius madagascarensis (Bonaparte, 1853)
	Pterythius melanotis Hodgson, 1855
M. inflatus Trouessart, 1884	*Caica leucogastra* (Kuhl, 1820)
	Ara s. severa (L. 1758)
	A. makawuanna (Gmelin, 1788)
	A. rauna Levaill, 1801
M. lyrioides Trouessart & Neumann, 1888	*Macropteryx mystacea* (Lesson, 1826)
M. lyrurus Trouessart & Neumann, 1888	*Rhytidoceros plicatus* (Forsten, 1781)
	Anorrhinus galeritus (Temminck, 1824)
	Buceros ruficollis Vieillot, 1816
	B. rhinoceros L. 1766
M. orientalis Trouessart, 1899	*Anthreptes malaccensis* (Scopoli, 1786)
M. similis Bonnet, 1924	*Cittocincla albicapilla* (Vieillot, 1818)
	Garrulax bellangeri Lesson, 1831
	G. schistochlamys Sharpe, 1888
	Eophona personata (Temminck & Schlegel, 1847)
	Melipotes gymnops Sclater, 1873
	Semnornis ramphastinus (Jardine, 1855)
M. spinosus Trouessart & Neumann, 1888	Bucerotidae
M. truncatipes Trouessart & Neumann, 1888	*Buceros rhinoceros* L. 1766
M. unilobatus Trouessart, 1899	*Cissa jeffreyi* Sharpe, 1888

Genus **Berlesella** Trouessart, 1919

B. alata Trouessart, 1919	*Chaetura* sp.

Genus **Megninia** Berlese, 1881

M. aequinoctialis Trouessart, 1899	*Phaethon aethereus* L. 1766
	P. rubricauda Boddaert, 1783
	P. phaenicurus Gmelin, 1788
	P. candidus Temminck, 1820
M. albida (Tyrrell, 1882)	*Tachycineta bicolor* (Vieillot, 1807)
M. aluconis (Buchholz, 1869)	*Syrnium aluco* (L. 1766)
M. analgoides Bonnet, 1924	*Petraeca leggi* Sharpe, 1879
	Malurus melanocephalus (Latham, 1801)
M. androgyna Trouessart, 1886	*Gallirallus a. australis* (Sparrmann, 1786)
	Strigops habroptilus Gray, 1845
	Nestor notabilis Gould, 1856
	Probosciger a. aterrimus (Gmelin, 1788)
M. angustella Bonnet, 1924	*Pitta caerulea* (Raffles, 1822)
M. bidentata Bonnet, 1924	*Paradisea sanguinea* Shaw, 1809
	P. rubra Daudin, 1800
M. brevitarsa Bonnet, 1924	*Meliphaga* sp.
	Melirophetes ochromelas Meyer, 1874
M. constricta Trouessart, 1899	*Aratinga solstitialis* (L. 1758)

M. cubitalis (Megnin, 1877)	*Meleagris g. gallopavo* L. 1758
	Phasianidae
M. c. var. *ginglymura* (Megnin, 1877)	Phasianidae
	Anatidae
	Corvidae
M. glandarii (Buchholz, 1869)	*Garrulus g. glandarius* (L. 1758)
M. gracillima Bonnet, 1924	*Zosterops madagascarensis* (Gmelin, 1788)
	Zosterops borbonica (Gmelin, 1789)
	Zosterops westernensis (Quoy & Gaimard, 1830)
	Zosterops clara Sharpe, 1888
	Vanga curvirostris (L. 1766)
	Chlorocharis emiliae Sharpe, 1888
	Pachycephala clio Wallace, 1862
M. hamata Bonnet, 1924	*Ibis bernieri* Bonaparte, 1850
M. harpipoda Trouessart, 1886	*Podargus papuensis* Quoy & Gaimard, 1824
M. ibidis Trouessart, 1885	*Plegadis f. falcinellus* (L. 1766)
	Platalea l. leucorodia L. 1758
M. i. var. *megacentrus* Trouessart, 1899	*Chibia carbonaria* Sharpe, 1877
M. laciniosa Bonnet, 1924	*Schistochlamys atra* (Gmelin, 1789)
	Pyroderus scutatus Shaw, 1792
M. laglaizeae Trouessart, 1886	*Falcinellus striatus* (Boddaert, 1783)
M. longipes Trouessart, 1899	*Palamedea cornuta* L. 1766
	Chaetura zonaris (Shaw, 1796)
	Hirundo senegalensis L. 1766
M. longitarsa Bonnet, 1924	*Nigrita canicapilla* (Strickland, 1841)
	N. bicolar (Hartlaub, 1844)
	Pyrenestes ostrinus (Vieillot, 1805)
M. manicata Trouessart, 1899	*Goura victoria* (Fraser, 1844)
M. megalixus Trouessart, 1885	*Cissa thalassina* (Temminck, 1826)
M. megacentros Trouessart, 1899	*Chibia carbonaria* Bonaparte, 1851
	Buchanga longicauda Hay, 1845
	Dicrurus forficatus (L. 1766)
M. micropus Trouessart, 1899	*Brachypteracias crossleyi* (Sharpe, 1877)
M. oedipus Trouessart, 1899	*Saurothera gigantea* Lafresnay
	Pelecanus rufescens Latham, 1785
	Coua cristata (L. 1766)
	Newtonia bruneicauda (Newton, 1863)
	Cacomantis virescens (Bruggeman, 1876)
	C. inspiratus (Gould, 1845)
	Calliecthrus leucolophus (Muller, 1839)
	Calcococcyx mayeri (Salvador, 1879)
M. ogivalis Trouessart, 1899	*Sula piscator* (L. 1766)
M. parallela Bonnet, 1924	*Lanius borealis* Vieillot, 1807
M. pedimana Trouessart, 1899	*Diomedea exulans* L. 1766
M. perforata Bonnet, 1924	*Lophotibis cristatus* (Boddaert, 1783)
M. remipes Trouessart, 1899	*Pelecanus fuscus* Gmelin, 1789
M. scapularis Trouessart, 1899	*Otidiphaps n. nobilis* Gould, 1870
M. strongylia Bonnet, 1924	*Poecilodryas hypoleuca* (Gray, 1859)
	P. albonotata (Salvador, 1875)
M. sublobata Trouessart, 1899	*Todopsis cyanocephala* (Quoy & Gaimard, 1830)
M. tetraonis Trouessart, 1899	*Tetrao tetrix* L. 1766
M. tumens Trouessart, 1899	*Psarisomus dalhousiae* (Jameson, 1835)

Genus **Pandalura** Hull, 1934

P. aestivalis (Berlese, 1883)	*Micropus apus apus* (L. 1758)
P. a. var. *subintegra* (Berlese, 1883)	*Martula u. urbica* (L. 1758)
	Riparia r. riparia (L. 1758)
P. grossa (Berlese, 1898)	*Gallinula c. chloropus* (L. 1758)

P. oscinum (Koch, 1840)	*Chloris chloris* (L. 1766)
	Cittocincla albicapilla (Vieillot, 1818)
	Geospiza strenua Gould, 1837
	Foudia eminentissima Bonaparte, 1850
	F. consobrinus Milne Edwards & Oustalet, 1885
	F. madagascarensis (L. 1766)
	Hemiphaga n. novaeselandiae (Gmelin, 1789)
	Chibia bracteata (Gould, 1842)
	Garrulax schistochlamys Sharpe, 1888
	G. bellangeri Lesson, 1831
	Pipreola riefferi (Boissoneau, 1840)
	P. viridis (Lafresnay & D'Orbigny, 1837)
	P. sclateri (Cornalia, 1852)
	Heliangelus strophianus (Gould, 1846)
	Poecilothraupis lunulatus (Du Bos, 1839)
	Muscivora tyrannus (L. 1766)
	Grallaria brevicauda (Vieillot, 1818)
P. pici-majoris (Buchholz, 1870)	*Picus viridis* L. 1758
	Dendrocopus m. major (L. 1758)
	Riparia r. riparia (L. 1758)
	Cotyle rupestris (Scopoli, 1769)
	Semnornis ramphastinus (Jardine, 1855)
	Cephalopterus ornatus Geoffroy St Hilaire, 1809
	Chaetura zonaris (Shaw, 1796)
	Corvus senex Lesson, 1828
	Rhipidura tricolor (Vieillot, 1818)
	Rhamphastos sulfuratus Lesson, 1830
	Chelidorhynx hypoxanthus (Blyth, 1843)
	Tephrodornis gularis (Raffles, 1822)
P. rallorum (Trouessart, 1885)	*Rallus a. aquaticus* L. 1758
P. strigis-oti (Buchholz, 1869)	*Asio otus otus* (L. 1758)

Genus **Diplaegidia** Hull, 1934

D. columbae (Buchholz, 1869)	*Columba domestica* L. 1758
	Serinus canarius (L. 1766)
D. gallinulae (Buchholz, 1869)	*Gallinula c. chloropus* (L. 1758)
	Porzana porzana (L. 1766)
	Rallus a. aquaticus L. 1758
	Vanellus vanellus (L. 1758)
	Coturnix c. coturnix (L. 1758)
D. major (Berlese, 1883)	*Porzana porzana* (L. 1766)

Genus **Leptosphyra** Hull, 1934

L. centropoda (Megnin, 1877)	*Vanellus vanellus* (L. 1758)
L. gracilipes (Trouessart & Neumann, 1888)	*Tringa totanus* (L. 1758)
L. strictior (Berlese, 1884)	*Tringa* sp.
L. velata (Megnin, 1877)	Anatidae

Genus **Ingrassia** Oudemans, 1905

I. veliger (Oudemans, 1904)	

Genus **Hemialges** Trouessart, 1888

H. anacentrus (Trouessart, 1899)	Pigeon
H. astrolabei Trouessart, 1919	*Pachycephala astrolabi* (Bonaparte, 1850)
H. attenuatus Trouessart, 1919	*P. poliosoma* (Sharpe, 1832)
H. circinipes (Trouessart, 1899)	*Paradisea rubra* Daudin, 1800
H. clypeata (Trouessart, 1899)	*Parotia sefilata* (Forsten, 1781)
H. curvispina Trouessart, 1919	*Pachycephala sororcula* De Vis, 1897
H. forcipes Trouessart, 1919	*Mystacornis crossleyi* (Grandidier, 1870)

H. furcula Trouessart, 1919	*Poecilodryas cyana* (Salvadori, 1874)
H. hologastra (Trouessart, 1899)	*Opopsitta d. diophthalma* (Hombron & Jacquinot, 1841)
H. humerosus Trouessart, 1919	*Eurylaimus javanicus* Horsfield, 1821
H. lativentris Trouessart, 1919	*Pachycephala flavifrons* (Peale, 1848)
H. longiventer Trouessart, 1919	*P. sororcula* De Vis, 1897
H. megamerus Trouessart, 1919	*P. leucostigma* Salvadori, 1875
H. mimus Trouessart, 1919	*Phonygama keraudreni* Lesson, 1826
H. pappus Trouessart & Neumann, 1888	*Manucodia atra* (Lesson, 1830)
H. pardalis Trouessart, 1919	*Pachycephala schlegeli* Schlegel, 1871
H. phanerorchis Trouessart, 1919	*Megalestes albonotatus* Salvadori, 1875
H. priapus Trouessart, 1919	*Pachycephala schlegeli* Schlegel, 1871
H. simulans Trouessart, 1919	*Orthonyx novae-guineae* Meyer, 1874
H. spinicrus Trouessart, 1919	*O. spinicauda* Temminck, 1827
H. spinosus Trouessart, 1919	*Terpsiphone mutata* (L. 1766)
H. strictolabatus Trouessart, 1919	*Megalestes albonotatus* Salvadori, 1875

Genus **Hyperalges** Trouessart, 1915

H. magnifica (Trouessart, 1895)	Paradiseidae
	Lophorina superba Tennant, 1790

Genus **Analges** Nitzsch, 1818

A. affinus Haller, 1877	*Tichodroma muraria* (L. 1766)
A. chelopus (Hermann, 1804)	Corvidae
	Paridae
	Motacillidae
A. cremidonotus Trouessart, 1899	*Pipilo megalonyx* Baird, 1858
A. digitatus Haller, 1882	*Dendroecia striata* (Forsten, 1772)
A. emarginatus Trouessart, 1899	*Hartlaubius madagascariensis* (Bonaparte, 1853)
A. hamatus Trouessart, 1899	*Ptilinopus pulchellus* (Temminck, 1835)
A. hoplophorus Trouessart & Neumann, 1888	*Mainatus robustus* (Salvadori, 1887)
A. integer Giebel, 1871	*Lanius e. excubitor* L. 1758
A. lobatus Trouessart, 1885	*Celeus e. elegans* (Muller, 1766)
A. longispinosus Tyrrell, 1882	*Calcarius nivalis* (L. 1766)
A. pullula Trouessart, 1899	*Humblotia flavirostris* A. Milne Edwards & Oustalet, 1885
A. spiniger Giebel, 1871	*Hippolais icterina* (Vieillot, 1817)
A. tetracentrus Trouessart, 1899	*Psephotus haematonotus* (Gould, 1837)
A. tyranni Tyrrell, 1882	*Tyrannus tyrannus* (L. 1758)

Genus **Analgopsis** Trouessart, 1919

A. bidentatus (Giebel, 1871)	*Prunelle modularis occidentalis* Hertert, 1910
A. bifidus (Nitzsch, 1818)	*Columba demestica* L. 1758
A. calcarata (Trouessart, 1886)	*Eutoxeres aquila* (Bourcier, 1847)
A. certhia (Haller, 1877)	*Certhia f. familiaris* L. 1758
A. corvinus (Megnin, 1877)	*Corvus c. corone* L. 1758
	Corvidae
A. mucronatus (Buchholz, 1869)	*Panurus biarmicus* (L. 1766)
	Paridae
A. pachycnemis (Giebel, 1871)	*Lanius e. excubitor* L. 1758
A. passerinus (L. 1758)	Passerines
A. pollicipatus (Trouessart, 1899)	*Coua caerulea* (L. 1758)
A. tridentulatus (Haller, 1882)	*Alauda a. arvensis* L. 1758

Genus **Plesialges** Trouessart, 1919

P. mimus Trouessart, 1919	*Pomathorinus* sp.

Genus **Protalges** Trouessart, 1885

Syn. *Hartingia* Oudemans, 1897	
P. accipitrinus Trouessart, 1885	*Falco t. tinnunculus* L. 1758
P. affinis Trouessart, 1886	*Pterophanes temmincki* (Boiss, 1839)
	Aglaeactis cupripennis (Bourc & Muls, 1843)
P. annulifer Trouessart, 1899	*Deroptyus a. accipitrinus* (L. 1758)
P. antipodum Trouessart, 1885	*Anthornis melanura* (Sparrmann, 1786)
P. attenuatus (Buchholz, 1869)	*Asio otus otus* (L. 1758)
	Strigidae
P. australis Trouessart, 1885	*Glyciphila fasciata* Gould, 1842
P. circiniger Trouessart, 1886	*Turacus buffoni* (Vieillot, 1819)
P. curtus Trouessart, 1885	*Platycercus e. elegans* (Gmelin, 1788)
P. intermedia Trouessart, 1899	*Aratinga solstitialis* (L. 1758)
P. lari (Oudemans, 1897)	Laridae
P. larva Trouessart, 1885	Psittacidae
P. longitarsus Trouessart & Neumann, 1887	*Petasophora iolata* Gould, 1847
	Eulampis jugularis (L. 1766)
P. lorinus Trouessart, 1885	*Domicella g. garrula* (L. 1758)
	D. domicella (L. 1758)
P. palmatus Trouessart, 1885	*Ortholophus leucolophus* (Sharpe, 1892)
P. psittacinus Trouessart, 1885	*Strigops habroptilus* Gray, 1845
P. robini Trouessart, 1885	*Aulacorhynchus s. sulcatus* (Swainson, 1820)

Genus **Nealges** Trouessart, 1886

N. poppei Trouessart, 1886	*Sula piscator* (L. 1766)

Genus **Pteralloptes** Trouessart & Megnin, 1884

Syn. *Analloptes* Trouessart, 1885	
P. bipartitus (Trouessart, 1885)	*Anthracoceros convexus* (Temminck, 1832)
	A. malayanus (Raffles, 1822)
P. corrugatus (Trouessart, 1885)	*Lophoceros leucolophus* (Sharpe, 1873)
	Anthracoceros malayanus (Raffles, 1822)
	Cranorrhinus corrugatus (Temminck, 1832)
P. elythrura (Trouessart, 1885)	Bucerotidae
P. falcinelli (Trouessart, 1885)	*Plegadis f. falcinellus* (L. 1766)
	Platalea l. leucorodia L. 1758
P. lyrura (Trouessart, 1899)	*Hemichelidon sibirica* (Gmelin, 1789)
P. megnini (Trouessart, 1885)	*Rallus a. aquaticus* L. 1758
P. pallens (Trouessart & Neumann, 1888)	*Porzana pusilla* (Pallas, 1776)
P. psophiae (Trouessart & Neumann, 1888)	*Psophia c. crepitans* L. 1758
P. pterocolurus (Trouessart, 1886)	*Tachypetes aquila* (L. 1766)
P. p. var. *velata* (Trouessart, 1886)	*T. aquila* (L. 1766)
P. stellaris (Buchholz, 1869)	*Botaurus s. stellaris* (L. 1758)
P. trifolium (Trouessart, 1899)	*Chera procne* (Boddaert, 1783)

Genus **Xolalges** Trouessart, 1885

X. analginus Trouessart, 1886	Rhamphastidae
	Dendroecia aestiva (Gmelin, 1788)
	Elainea martinica (L. 1766)
	Melirrhophetes leucostephes Meyer, 1874
X. astacopodus Trouessart & Neumann, 1888	*Chibia carbonaria* (Sharpe, 1877)
X. palliatus Trouessart, 1899	*Brachypteracias crossleyi* (Sharpe, 1877)
X. scaurus Trouessart, 1885	*Cuculus c. canorus* L. 1758
X. spinosus Trouessart & Neumann, 1888	*Todopsis cyanocephala* (Quoy & Gaimard, 1830)

Genus **Varchia** Oudemans, 1905

V. gambetta (Oudemans, 1904)	*Tringa flavipes* (Gmelin, 1788)

Genus **Bdellorhynchus** Trouessart, 1885

B. polymorphus Trouessart, 1885	*Anas c. crecca* L. 1758
	Spatula clypeata (L. 1758)
	Erismatura leucocephala (Scopoli, 1769)
B. psalidurus Trouessart, 1886	*Chenalopex aegyptiacus* (L. 1758)

Genus **Protolichus** Trouessart & Megnin, 1884

P. brachiatus Trouessart, 1884	*Domicella domicella* (L. 1758)
	Loriculus amabilis sclateri Wallace, 1862
	Trichoglossus h. haematod (L. 1771)
P. b. var. *crassior* Trouessart, 1884	*T. haematod moluccanus* (Gmelin, 1788)
	Glossopsitta concinna (Shaw, 1791)
	Loriculus amabilis sclateri Wallace, 1862
P. casuarinus Trouessart, 1884	*Chalcopsitta a. atra* (Scopoli, 1786)
P. chelidurus Trouessart, 1898	*Nannopsittaca panychlorus* (Salvin & Godman, 1883)
P. chiragricus Megnin & Trouessart, 1884	*Pezoporus formosus* (Latham, 1790)
	Platycercus caledonicus flaveolus Gould, 1837
	P. e. elegans (Gmelin, 1788)
P. eurycnemis Trouessart, 1884	*Ara macao* (L. 1758)
	Pyrrhura ferruginea (Muller, 1776)
P. falculiger Trouessart, 1884	*Glossopsitta concinna* (Shaw, 1791)
P. favettei Trouessart, 1898	*Nestor notabilis* Gould, 1856
	Nestor m. meridionalis (Gmelin, 1788)
	Northiella haematogaster haematorrhous Gould, 1865
P. furcatus Trouessart, 1895	*Coracopsis* sp.
P. hemiphyllus Megnin & Trouessart, 1884	*Bolbopsittacus l. lunulatus* (Scopoli, 1786)
P. lunula Robin, 1877	*Melopsittacus undulatus* (Shaw, 1805)
P. megamerus Trouessart, 1898	*Poicephalus eryptoxanthus zanzibaricus* Bowen, 1932
P. velifer Trouessart, 1884	*Eunymphicus c. cornutus* (Gmelin, 1788)
	Prosopeia personata (Gray, 1848)
	Platycercus caledonicus flaveolus Gould, 1837

Family FALCULIFERIDAE Oudemans, 1908

Genus **Falculifer** Railliet, 1896

Syn. *Falciger* Trouessart, 1885 pre-occ.	
F. cornutus (Trouessart, 1885)	*Cyanocorax violaceus* Du Bos, 1848
F. dinoceras (Trouessart, 1899)	*Geotrygon a. albifacies* (Sclater, 1858)
F. rostratus (Buchholz, 1869)	Columbidae
	Lophophaps p. plumifera (Gould, 1842)
	Goura coronata (L. 1758)
F. r. var. *longipes* (Trouessart, 1898)	*Trugon t. terrestris* Gray, 1849
	Megaloprepia magnifica puella (Lesson, 1827)
F. spinosus (Trouessart, 1898)	*Hemiphaga n. novaeselandiae* (Gmelin, 1789)

Genus **Chiloceras** Trouessart, 1898

Syn. *Cheiloceras*	
C. cervus Trouessart, 1898	*Calvenas n. nicobarica* (L. 1758)
	Trugon t. terrestris Gray, 1849
C. retusum Trouessart, 1898	*Myristicivora bicolor* (Scopoli, 1786)
C. taurus Trouessart, 1898	*Ducula p. pinon* (Quoy & Gaimard, 1824)
	Ducula goliath (Gray, 1859)

Genus **Anasicudiom** Trouessart & Neumann, 1888

A. landoisii (Buchholz, 1869)	*Buceros rhinoceros* L. 1766

Genus **Thecarthra** Trouessart, 1896

T. bouveti (Megnin & Trouessart, 1884)	*Charadrius hiaticula* L. 1758
T. interfolia (Megnin & Trouessart, 1884)	*Actitis hypoleucos* (L. 1758)
T. longitarsa (Megnin & Trouessart, 1884)	*Squatarola squatarola* (L. 1758)
	Pluvialis pluvialis (L. 1758)
T. setigera (Megnin & Trouessart, 1884)	*Limosa l. lapponica* (L. 1758)
T. simplex (Trouessart, 1886)	*Childonias n. nigra* (L. 1758)
T. s. var. *tyroglyphina* (Trouessart & Neumann, 1888)	*Hydrochelidon leucoptera* Schinz, 1815
T. theca (Megnin & Trouessart, 1884)	*Hydroprogne t. tschegrava* (Lepechin, 1770)
	Stercorarius parasiticus (L. 1758)
T. trouessarti Berlese, 1897	*Arenaria interpres* (L. 1758)

Genus **Sphaerogastra** Trouessart, 1897

S. monstrosa (Trouessart, 1898)	*Eclectus pectoralis* (Muller, 1764)
	Trichoglossus h. haematod (L. 1771)
S. thylacodes Trouessart, 1897	*Actitis littoreus* (L. 1766)
	Erolia testaceae (Pallas, 1764)

Genus **Neumanniella** Trouessart, 1915

Syn. *Neumannia* Trouessart & Neumann, 1888	
N. chelifer (Trouessart & Neumann, 1888)	*Crypturus cinnamomeus* (Lesson, 1842)

Genus **Syringobia** Trouessart & Neumann, 1888

S. calceata Trouessart, 1898	*Tringa ochropus* L. 1758
S. chelopus Trouessart & Neumann, 1888	*T. totanus* (L. 1758)
S. tricalcarata Trouessart & Neumann, 1888	*Charadrius dubius* Scopoli, 1786

Genus **Plutarchusia** Oudemans, 1904

P. chelopus (Trouessart & Neumann, 1888)	*Tringa totanus* (L. 1758)

Genus **Dermoglyphus** Megnin, 1877

D. concinnus Trouessart, 1898	*Ptilinopus purpuratus* (Gmelin, 1789)
D. deformis (Trouessart & Neumann, 1888)	*Schizoris concolor* (Smith, 1833)
D. diplectrum Trouessart, 1896	*Erolia testaceae* (Pallas, 1764)
D. elongatus (Megnin, 1877)	*Gallus domesticus* L. 1758
	Serinus canarius (L. 1766)
	Ploceidae
D. major (Trouessart, 1886)	*Eutoxeres aquila* (Bourcier, 1847)
	Phaethornis longirostris (Lesson & Delattre, 1843)
D. minor (Norner, 1882)	*Gallus domesticus* L. 1758
	Meleagris g. gallopavo L. 1758
	Numida m. meleagris (L. 1758)
D. paradoxus Trouessart, 1898	*Pyrrhura l. leucotis* (Kuhl, 1820)
	Conurus aeruginosus (L. 1766)
	Amazona f. farinosa (Boddaert, 1783)
D. protethes Trouessart, 1898	*Nelicurvius nelicourvi* (Scopoli, 1786)
D. pteronyssoides (Trouessart, 1886)	*Capella nigripennis* (Bonaparte, 1839)
	Aulacorhynchus caeruleicinctus d'Orbigny, 1840

Genus **Sammonica** Oudemans, 1904

S. ovalis (Trouessart, 1898)	*Tringa flavipes* (Gmelin, 1789)

Genus **Alloptes** Canestrini, 1879

A. abbreviatus Trouessart, 1885	*Plegadis ruber* (L. 1766)
A. alloptinus (Trouessart, 1886)	*Aglaeactis cupreipennis* (Bourcier & Muls, 1843)

A. aviculocaulis Trouessart, 1886	*Eutoxeres aquila* (Bourcier, 1847)
	Phaethornis longirostris (Lesson & Delattre, 1843)
	Trochilidae
A. crassipes (Canestrini, 1878)	*Limosa l. limosa* (L. 1758)
	Philomachus pugnax (L. 1758)
	Erolia a. alpina (L. 1758)
	Sterna minuta (L. 1758)
A. crassipes var. *conurus* Trouessart, 1885	Scolopacidae
	Laridae
A. c. var. *myosira* Trouessart, 1885	*Dromas ardeola* Paykull, 1805
A. c. var. *curtipes* Trouessart, 1886	*Haematopus ostralegus* L. 1758
	Actitis macularia macularia (L. 1766)
A. c. var. *minor* Trouessart, 1885	*Alca torda* L. 1758
	Fratercula a. arctica (L. 1758)
	Cepphus g. grylle (L. 1758)
	Larus ridibundus L. 1766
A. cypseli Canestrini & Berlese, 1881	*Apus a. apus* (L. 1758)
A. c. var. *longitarsa* Trouessart, 1899	*Chaetura zonarius* (Shaw, 1796)
A. dielytra Trouessart, 1885	*Pipra erythrocephala* L. 1758
	P. aureola L. 1758
A. discosurus Trouessart, 1886	*Podica senegalensis* (Vieillot, 1817)
A. euryurus Trouessart, 1885	*Platalea ajaja* L. 1766
A. fenestralis (Trouessart, 1885)	*Helianthea bonapartei* (Boiss, 1840)
A. intermedius (Trouessart & Neumann, 1888)	*Elainea martinica* (L. 1766)
	Loxigilla noctis (L. 1766)
A. lambda (Trouessart, 1885)	*Nettapus auritus* (Boddaert, 1783)
A. lobatus Trouessart, 1885	*Meliornis sericeus* (Gould, 1837)
A. lonchophorus Trouessart & Neumann, 1888	*Melirrhophetes leucostephes* Meyer, 1874
A. major (Trouessart, 1885)	*Menura superba* Davies, 1802
A. megamerus Trouessart, 1886	*Psittrichus pecquetii* (Lesson, 1831)
A. microphaethon Trouessart, 1885	*Phaethon aethereus* L. 1766
A. minutus Trouessart, 1899	*P. aethereus* L. 1766
	P. rubricauda Boddaert, 1783
A. norneri Trouessart, 1885	*Cyanolesbia mocoa* (Delattre & Bourcier, 1846)
	Trochilidae
A. petazophorus Trouessart, 1886	*Aramus s. scolopaceus* (Gmelin, 1789)
A. phaethontis (Gmelin, 1788)	*Phaethon rubricauda* Boddaert, 1783
	P. aethereus L. 1766
	Fratercula a. arctica (L. 1766)
A. p. var. *minor* Trouessart, 1885	*Phaethon aethereus* L. 1766
A. p. var. *simplex* Trouessart, 1885	*P. aethereus* L. 1766
A. pteronyssoides Trouessart, 1885	*Pipra aureola* L. 1758
	P. erythrocephala L. 1758
	Tyrannidae
A. quadrisetatus Berlese, 1885	*Ortygometra* sp.
A. scissus Trouessart, 1886	*Eutoxeres aquila* (Bourcier, 1847)
A. securiger Trouessart, 1885	*Dicaeum hirundinaceum* (Shaw & Nodder, 1792)
A. syringophorus Trouessart & Neumann, 1888	*Manucodia atra* (Lesson, 1830)
A. trogontia (Trouessart, 1885)	*Trogon collaris* (Vieillot, 1817)
	Harpactes orrhophaeus (Cabanis & Heine, 1863)

Genus **Monojoubertia** Radford, 1950

Syn. *Joubertia* Oudemans, 1905 pre occ.

M. aphyllus Trouessart, 1885	*Pinicola enucleator* (L. 1766)
M. flagellicaulus (Trouessart & Neumann, 1888)	*Capella g. gallinago* (L. 1758)

M. hemiphyllus (Robin, 1877)	*Fringilla c. coelebs* L. 1758
	F. montifringilla L. 1758
	Emberiza calandra L. 1758
M. microphyllus (Robin, 1877)	*Fringilla c. coelebs* L. 1758
M. modularis (Berlese, 1895)	*Prunella modularis occidentalis* Hertert, 1910

Genus **Trouessartia** Canestrini, 1899

Syn. *Pterocolus* Haller, 1878	
T. appendiculata (Berlese, 1884)	*Riparia r. riparia* (L. 1758)
	Micropus a. apus (L. 1758)
T. a. var. *minutipes* (Berlese, 1884)	*Martula u. urbica* (L. 1758)
T. bifurcata (Trouessart, 1885)	Sylviidae
	Eurylaemus ochromelas Raffles, 1822
	Centropus viridis (Scopoli, 1786)
T. caudacuta (Trouessart, 1899)	*Lobibyx lobatus* (Latham, 1801)
T. claviger (Trouessart, 1885)	*Plegadis ruber* (L. 1766)
T. coniventris (Trouessart, 1886)	*Tachypetes aquila* (L. 1766)
T. corvina (Koch, 1840)	Corvidae
T. eulabis (Buchholz, 1869)	*Mainatus javanensis* (Horsfield, 1820)
T. gracilepinnata (Haller, 1882)	*Empidonax flaviventris* (Baird, 1843)
T. rosteri (Berlese, 1883)	*Sturnus v. vulgaris* L. 1758
T. trachelura (Trouessart, 1885)	*Platalea ajaja* L. 1766

Genus **Pseudalges** Robin, 1868

Syn. *Allanalges* Trouessart, 1886	
P. acuticaudatus Trouessart, 1899	*Geoffroyus geoffroyi aruensis* (Gray, 1858)
P. analgoides (Trouessart, 1885)	*Merops apiaster* L. 1758
P. bifoliatus Trouessart & Neumann, 1888	*Megapodius f. freycineti* Gaimard, 1823
P. claudus Trouessart, 1899	*Brachypteracias pittoides* Lafresnaye, 1834
P. c. var. *anoma* Trouessart, 1899	*Philepitta jala* (Boddaert, 1783)
P. elegans (Trouessart, 1886)	*Pogoniulus bilineatus leucolaima* Verreaux, 1851
P. gracilipes (Trouessart, 1885)	*Lanius e. excubitor* L. 1758
	Psarisomus dalhousiae (Jameson, 1835)
	Cymbirhynchus macrorhynchus (Gmelin, 1788)
P. podagricus Trouessart, 1886	*Chrysococcyx smaragdineus* (Swainson, 1837)

Genus **Pterodectes** Robin, 1868

P. actidis (Canestrini, 1878)	*Actitis hypoleucos* (L. 1758)
	Tringidae
P. a. var. *discura* (Trouessart, 1885)	*Grus grus grus* (L. 1758)
P. bilaniatus (Trouessart, 1885)	*Mniotilta citrea* (Boddaert, 1783)
P. bilobatus Robin, 1868	*Anthus trivialis* (L. 1766)
	Alauda a. arvensis L. 1758
P. crassus Trouessart, 1885	*Cyanocorax chrysops* (Vieillot)
P. edwardsi (Trouessart, 1885)	*Acrocephalus arundinaceus* (L. 1758)
	Agrobates g. galactodes (Temminck, 1820)
P. gladiger Trouessart, 1885	Trochilidae
	Chrysolampis moschitus (L. 1758)
	Eulampis jugularis (L. 1766)
P. g. var. *hastifolia* Trouessart, 1899	*Clytolaema rubinea* (Gmelin, 1788)
P. gracilior Trouessart, 1885	*Topaza pella* (L. 1758)
	Chrysolampis moschitus (L. 1758)
	Lophornis ornatus (Boddaert, 1783)
	Trochilidae
P. gracilis Trouessart, 1885	*Ostinops decumanus* (Pallas, 1769)
	Xanthura yncas (Boddaert, 1783)
	Cyanocorax chrysops (Vieillot, 1818)
P. gracillimus Trouessart, 1886	*Ramphodon naevius* (Dumont, 1818)

P. gynurus (Trouessart, 1886)	*Chanelopex aegyptiacus* (L. 1758)
P. mainati Trouessart, 1869	*Maniatus javanensis* (Horsfield, 1820)
P. m. var. *aculeata* Canestrini, 1899	*Eurylaemus ochromelus* Raffles, 1822
	Lamprocolius glaucovirens Elliot, 1877
P. m. var. *trulla* Trouessart, 1885	*Turacus macrorhynchus* (Fraser, 1839)
P. m. var. *bacillus* Trouessart, 1885	*Ortygospiza polyzona* (Temminck, 1823)
P. manicatus Trouessart, 1885	*Glycyphila fasciata* Gould, 1842
P. megacaulus Trouessart, 1885	*Cinnyris afra* (L. 1766)
P. megalurus (Trouessart, 1899)	*C. notata* (Muller, 1716)
P. navicula Trouessart, 1899	*Nelicurvius nelicourvi* (Scopoli, 1786)
P. paradisiacus Trouessart, 1885	*Paradisea minor* Shaw, 1809
	Sericulus melinus (Latham, 1801)
P. phylloproctus Trouessart, 1886	*Podargus papuensis* Quoy & Gaimard, 1824
P. phyllurus Trouessart, 1899	*Manucodia atra* (Lesson, 1830)
P. p. var. *emarginata* Trouessart, 1899	*M. chalybata* (Forsten, 1781)
P. p. var. *diminuta* Trouessart, 1899	*M. atra* (Lesson, 1830)
P. p. var. *modesta* Trouessart, 1899	*M. atra* (Lesson, 1830)
P. rotifer (Trouessart & Neumann, 1888)	*Melirrhophetes leucostephes* Meyer, 1874
P. rutilus Robin, 1868	*Martula u. urbica* (L. 1758)
P. selenurus Trouessart, 1885	*Cyanolesbia mocoa* (Delattre & Bourcier, 1846)
	Topaza pella (L. 1758)
P. trochilidarum Trouessart, 1885	Trochilidae
	Chrysolampis moschitus (L. 1758)
	Topaza pella (L. 1758)
	Lophornis ornatus (Boddaert, 1783)
	Cyanolesbia mocoa (Delattre & Bourcier, 1846)
P. trouessarti Berlese, 1898	*Lanius e. excubitor* L. 1758
P. xiphiurus Trouessart, 1885	*Parisomus dalhousiae* (Jameson, 1835)

Genus **Montsauria** Oudemans, 1905

M. cylindricus (Robin, 1868)	*Pica pica pica* (L. 1758)

Genus **Favettea** Trouessart, 1915

F. heteroclyta Trouessart, 1915	*Spermospiza haematina* (Vieillot, 1805)

Family PROCTOPHYLLODIDAE Megnin & Trouessart, 1883

Genus **Proctophyllodes** Robin, 1868

P. ampelidis (Buchholz, 1869)	*Bombycilla garrula* (L. 1766)
	Prunella modularis occidentalis Hertert, 1910
	Anthidae
	Fringillidae
	Laniidae
	Corvidae
P. arcuaticaulis Trouessart, 1886	Acanthidae
P. attenuatus Trouessart, 1899	*Gymnostinops montezuma* (Lesson, 1830)
P. caulifer Trouessart, 1886	*Erithacus cyaneculus* (Wolf, 1810)
P. corvorum Vitzthum, 1922	Corvidae
P. cotyledon Trouessart, 1899	*Harporhynchus rediviva* (Gambel, 1845)
P. glandarinus (Koch, 1840)	Fringillidae
	Garrulus glandarius glandarius (L. 1758)
P. megaphyllus (Trouessart, 1885)	*Passer montanus* (L. 1758)
P. musicus Vitzthum, 1922	Turdidae
P. pennifer (Trouessart & Neumann, 1888)	*Notodela leucura* (Hodgson, 1845)
P. picae (Canestrini, 1886)	Passerines
P. pinnatus Vitzthum, 1922	Fringillidae
P. reticulifer (Trouessart & Neumann, 1888)	*Otocorys alpestris* (L. 1758)
P. sakatai Sugimoto, 1940	Drongo (*Dicrurus* sp.)

P. scolopacinus (Koch, 1840)	*Scolopax rusticola* L. 1758
P. securiclatus (Trouessart & Neumann, 1888)	*Melirrhophetes leucostephes* Meyer, 1874
P. stylifer (Buchholz, 1869)	Paridae
P. truncatus Robin, 1877	*Passer domesticus* (L. 1758)
	P. montanus (L. 1758)

Genus **Pterophagus** Megnin, 1877

P. strictus Megnin, 1877	Columbidae

Family EPIDERMOPTIDAE Trouessart, 1892

Genus **Epidermoptes** Rivolta, 1876

E. bilobatus Rivolta, 1876	*Gallus domesticus* L. 1758

Genus **Dermatium** Trouessart & Neumann, 1887

D. bihamatum (Trouessart & Neumann)	*Porzana pusilla* (Pallas, 1776)

Genus **Rivoltasia** Canestrini, 1894

R. bifurcata (Rivolta, 1876)	*Gallus domesticus* L. 1758
R. b. var. *major* Berlese, 1897	*Perdix rufa* (L. 1766)
R. dermicola (Trouessart, 1886)	*Passer d. domesticus* (L. 1758)
R. latior Canestrini, 1894	*Garrulus g. glandarius* (L. 1758)

Genus **Dermatophagoides** Bogdanow, 1864

Syn. *Pachylichus* Canestrini, 1894	
D. crassus Canestrini, 1894	*Luscinia phoenicurus* (L. 1766)

Genus **Microlichus** Trouessart & Neumann, 1887

M. avus (Trouessart, 1887)	*Gallinago major* (Gmelin, 1788)
	Capella nigripennis Bonaparte, 1839
	Dendrocopus medius (L. 1758)
	Garrulus g. glandarius (L. 1758)
	Eulampis holosericeus (L. 1758)
	Passer d. domesticus (L. 1758)
M. perdicis Canestrini, 1894	*Perdix p. perdix* (L. 1758)
M. uncus Vitzthum, 1934	*Ornithomyia fringillina*

Family HETEROPSORIDAE Oudemans, 1908

Genus **Heteropsorus** Trouessart & Neumann, 1887

H. pteroptopus Trouessart & Neumann, 1887	*Acrocephalus streperus* (Vieillot, 1817)
	Erithacus cyaneculus (Wolf, 1810)
	Emberiza cirlus L. 1766
	E. schoeniclus (L. 1766)

HOST LIST

CORVIDAE

Corvus corax corax L. 1758	Raven
C. cornix cornix L. 1758	Hooded Crow
C. corone corone L. 1758	Carrion Crow
C. f. frugilegus L. 1758	Rook
C. scapulatus Daudin, 1800	Pied Crow
C. senex Lesson, 1828	New Guinea Crow
Corone orru (Bonaparte, 1851)	New Guinea large-billed Crow
Macrocorax fuscicapillus (Gray, 1859)	Aru Crow
Glaucopis cinereas Gmelin, 1788	Orange-wattled Crow
Corvultur albicollis (Latham, 1790)	White-necked Raven

Pica pica pica (L. 1758)	Magpie
Cyanocorax chrysops (Vieillot, 1818)	Urraca Jay
Pyroderus scutatus Shaw, 1792	Scutated Fruit Crow
Cyanocorax violaceus Du Bus, 1848	Violaceus Jay
Garrulus gl. glandarius (L. 1758)	Continental Jay
Xanthura yncas (Boddaert, 1783)	Green Jay
Cissa jeffreyi Sharpe, 1888	Cissa
C. thalassina (Temminck, 1826)	Short-tailed Cissa
Heteralocha gouldi Cabanis, 1850	Huia
PARADISEIDAE	
Astrapia nigra (Gmelin, 1788)	Gorget Bird of Paradise
Paradisea minor Shaw, 1809	Lesser Bird of Paradise
P. rubra Daudin, 1800	Red Bird of Paradise
Parotia sefilata (Forster, 1781)	Six-plumed Bird of Paradise
Lophorina superba (Pennant, 1790)	Superb Bird of Paradise
Manucodia atra (Lesson, 1830)	Black Papuan Paradise Bird
M. chalybata (Forster, 1781)	Green Manucode
Phonygama keraudreni Lesson, 1826	Keraudren's Manucode
Sericulus melinus (Latham, 1801)	Yellow-bellied Thrush
Falcinellus striatus (Boddaert, 1783)	Great Sicklebill
STURNIDAE	
Sturnus v. vulgaris L. 1758	Starling
Hartlaubius madagascariensis (Bonaparte, 1853)	Madagascar Starling
EULABETIDAE	
Mainatus javanensis (Horsfield, 1820)	Grackle
M. robustus (Salvadore, 1887)	Nias Island Grackle
Lamprocolius glaucovirens Elliot, 1877	Splendid Glossy Starling
Calornis chalybea (Horsfield, 1820)	Glossy Starling
C. panayensis (Scopoli, 1783)	Philippine Glossy Starling
DICRURIDAE	
Dicrurus forficatus (L. 1766)	Madagascar Crested Drongo
Chibia carbonaria (Bonaparte, 1851)	New Guinea Spangled Drongo
C. bracteata (Gould, 1842)	Australian Drongo
Buchanga longicauda (Hay, 1845)	Indian Grey Drongo
PRIONOPIDAE	
Pinarolestes heinei (Finoch & Hartlaub, 1869)	Tonga Uniform Shrike Thrush
Tephrodornis gularis (Raffles, 1822)	Wood Shrike
Melanornectes nigrescens (Schlegel, 1871)	Wood Shrike
Rhectes cirrhocephalus (Lesson & Garnott, 1827)	Wood Shrike
Pitohui arvensis nigripectis (Van Oert, 1909)	Wood Shrike
ORIOLIDAE	
Oriolus striatus Quoy & Gaimard, 1830	New Guinea Oriole
ICTERIDAE	
Ostinops decumanus (Pallas, 1769)	Crested Cassique
Gymnostinops montezuma (Lesson, 1830)	Hangnest
PLOCEIDAE	
Munia maja (L. 1766)	Malacca Grosbeak
Nelicurvius nelicourvi (Scopoli, 1786)	Pensile Grosbeak
Chera procne (Boddaert, 1783)	Long-tailed Whydah
Ortygospiza polyzona (Temminck, 1823)	South African Quail Finch

Steganura paradisea (L. 1766)	Pin-tailed Whydah
Eophona personata (Temminck & Schlegel, 1847)	Japanese Hawfinch
Foudia consobrinus Milne-Edwards & Oustalet, 1885	Grand Comoro Fody
F. eminentissima Bonaparte, 1850	Johanna Fody
F. madagascarensis (L. 1766)	Madagascar Fody
Spermospiza haemitina (Vieillot, 1805)	Blue-billed Weaver
Tanagridae	
Schistochlamys atra (Gmelin, 1789)	Black-faced Tanager
Poecilothraupis lunulatus (Du Bus, 1839)	Scarlet-necked Tanager
Mniotiltidae	
Mniotilta citrea (Boddaert, 1783)	Wood Warbler
Dendroecia aestiva (Gmelin, 1788)	Green Warbler
D. striata (Forsten, 1772)	Blackpoll Warbler
Fringillidae	
Fringilla c. coelebs L. 1758	Continental Chaffinch
F. montifringilla L. 1758	Brambling
Passer domesticus domesticus (L. 1758)	House Sparrow
P. montanus (L. 1758)	Tree Sparrow
Pinicola enucleator (L. 1766)	Crested Bullfinch
Emberiza calandra L. 1758	Corn Bunting
E. cirlus L. 1766	Cirl Bunting
E. schoeniclus (L. 1766)	Reed Bunting
Pipilo megalonyx Baird, 1858	San Diego Towhee
Serinus canarius (L. 1766)	Canary
Chloris chloris (L. 1766)	Greenfinch
Loxigilla noctis (L. 1766)	Black Sparrow
Calcarius nivalis (L. 1766)	Snow Bunting
Geospiza strenua Gould, 1837	Long-billed Ground Finch
Nigrita bicolor (Hartlaub, 1844)	Chestnut-breasted Negro Finch
N. canicapilla (Strickland, 1841)	Grey-headed Negro Finch
Pyrenestes ostrinus (Vieillot, 1845)	Black-headed Seedcracker
Alaudidae	
Alauda arvensis arvensis L. 1758	Skylark
Otocorys alpestris (L. 1758)	Northern Horned Lark
Motacillidae	
Anthus trivialis (L. 1766)	Tree Pipit
Certhiidae	
Certhia familiaris familiaris L. 1758	Northern Tree Creeper
Tichodroma muraria (L. 1766)	Wall Creeper
Meliphagidae	
Glycyphila fasciata Gould, 1842	White-breasted Honey Eater
Melirophetes ochromelas Meyer, 1874	Ochraceous Honey Eater
M. leucostephes Meyer, 1874	New Guinea Honey Eater
Melipotes gymnops Sclater, 1873	Naked-faced Honey Eater
Melilestes megarhynchus (Gray, 1858)	Large-billed Honey Eater
Meliornis sericeus (Gould, 1837)	New Holland Creeper
Anthornis melanura (Sparrman, 1786)	Rocking Creeper
Zosteropidae	
Zosterops borbonica (Gmelin, 1789)	Bourbon Manioc Bird
Z. clara Sharpe, 1888	Black-capped White Eye

Z. madagascarensis (Gmelin, 1788)	Madagascar White Eye
Z. westernensis (Quoy & Gaimard, 1830)	Grey-breasted White Eye
Chlorocharis emiliae Sharpe, 1888	Sharpe's White Eye
NECTARINIIDAE	
Anthreptes malaccensis (Scopoli, 1786)	Brown-throated Sunbird
Cinnyris afra (L. 1766)	Double-collared Sunbird
C. notata (Muller, 1766)	Madagascar Green Sunbird
DICAEIDAE	
Dicaeum hirundinaceum (Shaw & Nodder, 1792)	Flower Pecker
PARIDAE	
Parus caeruleus L. 1760	Blue Titmouse
P. cristatus L. 1761	Crested Titmouse
PANURIDAE	
Panurus biarmicus (L. 1766)	Bearded Titmouse
LANIIDAE	
Lanius e. excubitor L. 1758	Great Grey Shrike
L. borealis Vieillot, 1807	Northern Shrike
Grallina picata (Latham, 1801)	Magpie Lark
Pachycephala astrolabi Bonaparte, 1850	Solomon Islands Thickhead
P. clio Wallace, 1862	Large-billed Thickhead
P. flavifrons (Peale, 1848)	Yellow-fronted Thickhead
P. leucostigma Salvadori, 1875	Arfak Mountains Thickhead
P. poliosoma (Sharpe, 1832)	Grey Thickhead
P. schlegeli Schlegel, 1871	Schlegel's Thickhead
P. sororcula Du Vis, 1897	Owen Stanley Thickhead
Vanga curvirostris (L. 1766)	Hook-billed Vanga
AMPELIDAE	
Bombycilla garrula (L. 1766)	Waxwing
TURDIDAE	
Cittocincla albicapilla (Vieillot, 1818)	White-crowned Robin Chat
Prunella modularis occidentalis Hartert, 1910	Hedge Sparrow
Luscinia suecica cyanecula (Meisner, 1804)	White-spotted Bluethroat
L. m. megarhyncha Brehm, 1831	Nightingale
L. phoenicurus (L. 1766)	Redstart
Erithacus cyaneculus (Wolf, 1810)	White-spotted Bluethroat
Grallaria brevicauda (Vieillot, 1818)	Short-tailed Ant-Thrush
SYLVIIDAE	
Sylvia atricapilla atricapilla (L. 1758)	Black Cap
Agrobates g. galactodes (Temminck, 1820)	Rufous Warbler
Hippolais icterina (Vieillot, 1817)	Icterine Warbler
Acrocephalus a. arundinaceus (L. 1758)	Great Reed Warbler
A. s. scirpaceus (Hermann, 1804)	Reed Warbler
A. streperus (Vieillot, 1817)	Reed Warbler
MUSCICAPIDAE	
Muscicapa striata (Pallas, 1764)	Spotted Flycatcher
Terpsiphone mutata (L. 1766)	Madagascar Paradise Flycatcher
Empidonax flaviventris (Baird, 1843)	Yellow-bellied Flycatcher
Chelidorhynx hypoxanthus (Blyth, 1843)	Yellow-bellied Flycatcher
Todopsis cyanocephala (Quoy & Gaimard, 1830)	New Guinea Flycatcher

Humblotia flavirostris Milne-Edwards & Oustalet, 1885	Humblot's Flycatcher
Muscivora tyrannus (L. 1766)	Swallow-tailed Flycatcher
Rhipidura tricolor (Vieillot, 1818)	Black and White Fantailed Flycatcher
Hemichelidon sibirica (Gmelin, 1789)	Dun Flycatcher
Megarhynchus pitangua (L. 1766)	Boat-billed Flycatcher
Poecilodryas albonotata (Salvadori, 1875)	Black-throated Fly Robin
P. cyana (Salvadori, 1874)	Blue Fly Robin
P. hypoleuca (Gray, 1859)	White-breasted Fly Robin
Megalestes albonotatus Salvadori, 1875	Black-throated Fly Robin
Petraeca leggi Sharpe, 1879	Tasmanian Scarlet-breasted Robin

HIRUNDINIDAE

Hirundo senegalensis L. 1766	Mosque Swallow
Martula urbica urbica (L. 1758)	House Martin
Riparia r. riparia (L. 1758)	Sand Martin
Cotyle rupestris (Scopoli, 1769)	European Crag Martin
Tachycineta bicolor (Vieillot, 1807)	Tree Swallow

TIMELIIDAE

Pterythius melanotis Hodgson, 1855	Chestnut-throated Shrike Babbler
Garrulax bellangeri Lesson, 1831	Burmese White-crested Laughing Thrush
G. schistochlamys Sharpe, 1888	Grey and Brown Laughing Thrush
Mystacornis crossleyi (Grandidier, 1870)	Crossley's Babbler
Orthonyx novae-guineae Meyer, 1874	Papuan Orthonyx
O. spinicauda Temminck, 1827	Spine-tailed Orthonyx
Notodela leucura (Hodgson, 1845)	White-tailed Blue Robin
Harporhynchus rediviva (Gambel, 1845)	Californian Thrasher
Malurus melanocephalus (Latham, 1801)	Red-backed Wren
Conostoma oemodium Hodgson, 1841	Great Parrot-billed Babbler

TYRANNIDAE

Elainea martinica (L. 1766)	Antillean Elainea
Tyrannus tyrannus (L. 1758)	Tyrant Bird

COTINGIDAE

Cephalopterus ornatus Geoffroy Saint-Hilaire, 1809	Umbrella Bird

PIPRIDAE

Pipra aureola L. 1758	Orange-headed Mannakin
P. erythrocephala L. 1758	Golden-headed Mannakin
Cirrhopipra filicauda (Spix, 1825)	Cirrhate Mannakin
Pipreola riefferi (Boissonneau, 1840)	Reiffer's Fruit Eater
P. sclateri (Cornalia, 1852)	Sclater's Fruit Eater
P. viridis (Lafresnaye & D'Orbigny, 1837)	D'Orbigny's Fruit Eater

EURYLAEMIDAE

Eurylaemus javanicus Horsfield, 1821	Banded Broadbill
E. ochromelas Raffles, 1822	Black and Yellow Broadbill
Psarisomus dalhousiae (Jameson, 1835)	Long-tailed Broadbill
Cymbirhynchus macrorhynchus (Gmelin, 1788)	Black and Yellow Broadbill

PITTIDAE

Pitta caerulea (Raffles, 1822)	Giant Pitta
Philepitta jala (Boddaert, 1783)	Velvet Pitta

DENDROCOLAPTIDAE

Furnarius torridus Sclater & Salvin, 1866	Pale-bellied Oven Bird

MENURIDAE	
Menura superba Davies, 1802	Lyre Bird
PICIDAE	
Celeus e. elegans (Muller, 1776)	Elegant Crested Woodpecker
Picus canus (Gmelin, 1788)	Grey-headed Grey Woodpecker
P. viridis L. 1758	Green Woodpecker
Melanerpes e. erythrocephalus (L. 1758)	Red-headed Woodpecker
Dendrocopus major major (L. 1758)	Greater Spotted Woodpecker
D. medius (L. 1758)	Spotted Woodpecker
D. villosus (L. 1766)	Hairy Woodpecker
CAPITONIDAE	
Capito niger niger (Muller, 1776)	Cayenne Barbet
C. n. auratus (Dumont, 1816)	Golden-fronted Barbet
Semnornis rhamphastinus (Jardine, 1855)	Toucan Barbet
Lybius b. bidentatus (Shaw, 1798)	Double-toothed Barbet
Pogoniulus bilineatus leucolaima (Verreaux, 1851)	Lemon-rumped Tinker Bird
RHAMPHASTIDAE	
Aulacorhynchus s. sulcatus (Swainson, 1820)	Groove-billed Toucanet
A. caerulei-cinctus Orbigny, 1840	Blue-banded Toucanet
Rhamphastos dicolorus L. 1766	Green-billed Toucan
R. s. sulfuratus Lesson, 1830	Keel-billed Toucan
Pteroglossus a. aracari (L. 1758)	Aracari Toucan
TROGONIDAE	
Trogon collaris Vieillot, 1817	Trogon
Harpactes orrhaphaeus (Cabanis & Heine, 1863)	Cinnamon-rumped Trogon
COLIIDAE	
Colius nigricollis Vieillot, 1817	Black-necked Coly
TROCHILIIDAE	
Rhamphodon naevius (Dumont, 1818)	Sawbill
Eulampis holosericeus (L. 1758)	Green Carib
E. holosericeus (L. 1758)	Purple Carib
Petasophora iolata Gould, 1847	Gould's Violet-ear
Chrysolampis moschitus (L. 1758)	Ruby-Topaz Humming Bird
Pterophanes temmincki (Boiss, 1839)	Temminck's Sapphire-wing
Helianthea bonapartei (Boiss, 1840)	Bonaparte's Star-frontlet
Cyanolesbia mocoa (Delattre & Bourcier, 1846)	Green-tailed Sylph
Phaethornis longirostris (Lesson & Delattre, 1843)	Long-billed Hermit
Clytolaema rubinea (Gmelin, 1788)	Brazilian Ruby
Eutoxeres aquila (Bourcier, 1847)	Curved-beaked Hermit
Topaza pella (L. 1758)	Crimson Topaz
Aglaeactis cupreipennis (Bourcier & Muls. 1843)	Shining Sunbeam
Lophornis ornatus (Boddaert, 1783)	Tufted Coquett
Heliangelus strophianus (Gould, 1846)	Gorgeted Sun Angel
CYPSELIDAE	
Micropus apus apus (L. 1758)	Swift
M. melba (L. 1758)	Alpine Swift
Chaetura zonaris (Shaw, 1796)	Needle-tailed Swift
Macropteryx mystacea (Lesson, 1826)	Bearded Tree Swift
MEROPIDAE	
Merops apiaster L. 1758	Bee Eater

BUCEROTIDAE	
Buceros plicatis Forster, 1781	Wreathed Hornbill
B. rhinoceros L. 1766	Rhinoceros Hornbill
B. ruficollis Vieillot, 1816	Hornbill
Dichoceros bicornis (L. 1766)	Great Pied Hornbill
Ortholophus leucolophus (Sharpe, 1892)	Gold Coast White-crested Hornbill
Lophoceros erythrorhynchus (Temminck, 1823)	Red-billed Hornbill
L. melanoleucus (Lichtenstein, 1793)	Yellow-beaked Hornbill
Penelopides manillae (Boddaert, 1783)	Manilla Hornbill
Anorrhinus galeritus (Temminck, 1824)	Malay Wedge-tailed Hornbill
Hydrocorax hydrocorax (L. 1766)	Phillipine Hornbill
Anthracoceros convexus (Temminck, 1832)	Pied Hornbill
A. coronatus (Boddaert, 1783)	Pied Hornbill
A. malabaricus (Gmelin, 1788)	Pied Hornbill
Rhinoplax vigil (Forster, 1781)	Helmeted Hornbill
Anthracoceros malayanus (Raffles, 1822)	Black Hornbill
Cranorrhinus corrugatus (Temminck, 1832)	Wrinkled Hornbill
C. leucocephalus (Vieillot, 1816)	White-headed Hornbill
Rhytidoceros plicatus (Forster, 1781)	Wreathed Hornbill
ALCEDINIDAE	
Alcyone azurea (Latham, 1826)	Kingfisher
CORACIIDAE	
Brachypteracias crossleyi (Sharpe, 1877)	Crossley's Ground Roller
B. pittoides Lafresnaye, 1834	Pitta-like Ground Roller
PODARGIDAE	
Podargus papuensis Quoy & Gaimard, 1824	Papuan Frogmouth
P. strigoides (Latham, 1801)	Great-headed Frogmouth
CUCULIDAE	
Cuculus canorus canorus L. 1758	Cuckoo
Chrysococcyx smaragdineus (Swainson, 1837)	Emerald Cuckoo
Centropus viridis (Scopoli, 1786)	Red-winged Coucal
Coua caerulea (L. 1758)	Blue Cuckoo
C. cristata (L. 1766)	Madagascar Crested Coucal
Saurothera gigantea Lafresnay	Cuban Lizard Cuckoo
Cacomantis insperatus (Gould, 1845)	Brush Cuckoo
C. virescens (Bruggeman, 1876)	Cuckoo
Calliechthrus leucolophus (Muller, 1839)	White-crowned Black Cuckoo
Calcococcyx meyeri (Salvadori, 1874)	Meyer's Golden Cuckoo
MUSOPHAGIDAE	
Turacus buffoni (Vieillot, 1819)	Senegal Lourie
T. macrorhynchus (Fraser, 1839)	Black-tip Crested Lourie
T. meriani (Ruppel, 1851)	Red-tip Crested Lourie
Musophaga violacea Isert, 1789	Senegal Violet Plantain Eater
Schizoris africana (Latham, 1789)	Grey Plantain Eater
S. concolor (Smith, 1833)	Goaway Bird
NESTORINAE	
Nestor m. meridionalis (Gmelin, 1788)	Kaka
N. notabilis Gould, 1856	Kea
LORIINAE	
Chalcopsitta atra atra (Scopoli, 1786)	Lory
Trichoglossus h. haematod (L. 1771)	Red-breasted Parakeet

T. h. moluccanus (Gmelin, 1788)	Swainson's Lorikeet
T. rubiginosus (Bonaparte, 1850)	Red-winged Lory
Domicella domicella (L. 1758)	Purple-capped Lory
D. g. garrula (L. 1758)	Scarlet Lory
Coryphilus taitianus (Gmelin, 1788)	Otahetian Blue Parakeet
Glossopsitta concinna (Shaw, 1791)	Pacific Parrot
Opopsitta d. diophthalma (Hombron & Jacquinot, 1841)	Parrot
KAKATOEINAE	
Probosciger a. aterrimus (Gmelin, 1788)	Great Black Cockatoo
Calyptorhynchus magnificus macrorhynchus Gould, 1842	Raven Cockatoo
PSITTACINAE	
Ara canga Levaill, 1801	Macaw
A. chloroptera Gray, 1859	Red and Yellow Macaw
A. macao (L. 1758)	Red and Blue Macaw
A. makawuana (Gmelin, 1788)	Parrot Macaw
A. nobilis nobilis (L. 1758)	Noble Parrot
A. rauna Levaill, 1801	Blue and Yellow Macaw
A. severa severa (L. 1758)	Brazilian Green Macaw
Conurus aeruginosus (L. 1766)	Brown-throated Parakeet
C. c. canicularis (L. 1758)	Red and Blue-headed Parakeet
Aratinga solstitialis (L. 1758)	Golden Parakeet
Nandayus nenday (Vieillot, 1823)	Hooded Parrot
Ognorhynchus icterotis (Massena & Souance, 1854)	Yellow-eared Parakeet
Pyrrhura cruentata (Wied, 1820)	Sanguine Parakeet
P. ferruginea (Muller, 1776)	Emerald Parakeet
P. l. leucotis (Kuhl, 1820)	White-eared Parakeet
P. picta picta (Muller, 1776)	Scaly-breasted Parakeet
Forpus p. passerinus (L. 1758)	Blue-winged Parakeet
Brotogeris versicolurus chiriri (Vieillot, 1817)	Green Parakeet
Nannopsittaca panychloris (Salvin & Godman, 1883)	Roraima Parakeet
Pionus menstruus (L. 1766)	Blue-headed Parrot
Amazona f. farinosa (Boddaert, 1783)	Amazon Parrot
Poicephalus cryptoxanthus zanzibaricus Bowen, 1932	Brown-headed Parrot
P. gulielmi (Jardine, 1849)	Congo Red-headed Parrot
Coracopsis vasa comorensis (Peters, 1854)	Comoran Parrot
Psittrichus pecquetii (Lesson, 1831)	Papuan Black Parrot
Deroptyus a. accipitrinus (L. 1758)	Hawk-billed Parrot
Ptilinopus purpuratus (Gmelin, 1789)	Purple-tailed Parakeet
Caica leucogastra Kuhl, 1820	White-bellied Caigoe
C. melanocephala (L. 1766)	White-breasted Parrot
Nasiterna pygmae (Quoy & Gaimard, 1830)	Pygmy Parrot
Lorius roratus pectoralis (Muller, 1776)	Red-flanked Parrot
Geoffroyus g. aruensis (Gray, 1858)	Geoffroy's Parrot
Prosopeia personata (Gray, 1848)	Fiji Parrot
Bolbopsittacus l. lunulatus (Scopoli, 1786)	Collared Parakeet
Loriculus amabilis sclateri Wallace, 1862	Sclater's hanging Parrot
Platycercus e. elegans (Gmelin, 1788)	Broadtail
P. caledonicus flaveolus Gould, 1837	Yellow Parrot
Psephotus haematonotus (Gould, 1837)	Red-backed Parrot
Northiella haematogaster haematorrhous (Gould, 1865)	Crimson-bellied Parakeet
Eunymphicus c. cornutus (Gmelin, 1788)	New Caledonian Parakeet
Melopsittacus undulatus (Shaw, 1805)	Budgerigar
Pezoporus formosus (Latham, 1790)	Long-tailed Swamp Parakeet
STRIGOPINAE	
Strigops habroptilus Gray, 1845	Kakapo

STRIGIDAE	
Strix aluco sylvatica Shaw, 1809	Tawny Owl
S. nebulosum (Forster, 1772)	Barred Owl
BUBONIDAE	
Syrnium aluco (L. 1766)	Tawny Owl
Carine passerina (L. 1758)	Little Owl
Asio otus otus (L. 1758)	Long-eared Owl
Otus scops scops (L. 1758)	Scops Owl
PANDIONIDAE	
Pandian haliaetus haliaetus (L. 1758)	Osprey
Falco subbuteo subbuteo L. 1758	Hobby
F. t. tinnunculus L. 1758	Kestrel
Pernis apivorus apivorus (L. 1758)	Honey Buzzard
Milvus milvus milvus (L. 1758)	Kite
Haliastur indus girrenera (Vieillot, 1822)	Brahmini Kite
Haliaetus leucogaster (Gmelin, 1788)	White-bellied Sea Eagle
Circaetus gallicus (Gmelin, 1788)	Short-toed Eagle
Aquila chrysaetus (L. 1758)	Golden Eagle
A. pomarina Brehm, 1831	Rough-footed Eagle
Harpyia destructor Cuvier, 1817	Harpy Eagle
Buteo buteo buteo (L. 1758)	Common Buzzard
Accipiter nisus nisus (L. 1758)	Sparrow Hawk
Circus pygargus (L. 1758)	Montagu's Harrier
PHALACROCORACIDAE	
Bugeranus aristotelis aristotelis (L. 1761)	Shag
B. carunculatus (Gmelin, 1789)	Cormorant
Francolinus l. levaillantii (Valenciennes, 1825)	African Darter
SULIDAE	
Sula bassana (L. 1758)	Gannet
S. fiber (L. 1766)	Lesser Gannet
S. piscator (L. 1766)	Gannet
PELECANIDAE	
Pelecanus fuscus Gmelin, 1789	West Indian Brown Pelican
P. rufescens Latham, 1785	Red-backed Pelican
FRIGATIDAE	
Tachypetes aquila (L. 1766)	Great Frigate Bird
PHAETONTIDAE	
Phaethon aethereus L	Red-beaked Tropic Bird
P. candidus Temminck, 1820	White-tailed Tropic Bird
P. phaenicurus Gmelin, 1788	Red-tailed Tropic Bird
P. rubricauda Boddaert, 1783	Tropic Bird
ANATIDAE	
Cygnus olor (Gmelin, 1789)	Mute Swan
Nettapus auritus (Boddaert, 1783)	Madagascar Teal
Anser anser anser (L. 1758)	Grey Lag Goose
A. h. hyperboreus (Pallas, 1769)	Snow Goose
Chenalopex aegyptiacus (L. 1758)	Knob-winged Goose
Erismatura leucocephala (Scopoli, 1769)	White-headed Goose
Anas acuta acuta L. 1758	Pintail
A. crecca crecca L. 1758	Teal

A. querquedula L. 1758	Garganey
Spatula clypeata (L. 1758)	Shoveller
Aythya fuligula (L. 1758)	Tufted Duck
Mergus serrator L. 1758	Red-breasted Merganser
PHOENICOPTERIDAE	
Phoenicopterus ruber roseus Pallas, 1811	European Flamingo
PALAMEDEIDAE	
Palamedea cornuta L. 1766	Horned Screamer
ARDEIDAE	
Aramus s. scolopaceus (Gmelin, 1789)	Scolopaceus Heron
Botaurus stellaris stellaris (L. 1758)	Bittern
SCOPIDAE	
Scopus umbretta Gmelin, 1788	Hammerhead
CICONIDAE	
Ciconia ciconia ciconia (L. 1758)	White Stork
C. nigra (L. 1758)	Black Stork
Euxenura maguari (Gmelin, 1788)	Magwari Stork
Ephippiorhynchus senegalensis (Shaw, 1798)	Saddle-backed Stork
Leptoptilis argala Gray, 1844	Maribou Stork
Tantalis ibis L. 1766	African Wood Stork
IBIDIDAE	
Ibis melanocephala (Latham, 1787)	Black-headed Ibis
I. berneiri Bonaparte, 1850	Madagascar Sacred Ibis
I. molucca Cuvier, 1829	White Ibis
Plegadis falcinellus falcinellus (L. 1766)	Glossy Ibis
P. ruber (L. 1766)	Scarlet Ibis
Lophotibis cristatus (Boddaert, 1783)	White-winged Ibis
Theristicus caruncula tus (Ruppel, 1835)	Wattled Ibis
T. leucocephalus Reich, 1894	Hagedash
T. melanopis (Gmelin, 1788)	Black-faced Ibis
PLATALEIDAE	
Platalea ajaja L. 1766	Roseate Spoonbill
P. l. leucorodia L. 1758	Spoonbill
JACANIDAE	
Hydrophasianus chirurgus (Scopoli, 1786)	Chinese Jacana
HAEMATOPODIDAE	
Haematopus ostralegus L. 1758	Oystercatcher
CHARADRIIDAE	
Vanellus vanellus (L. 1758)	Lapwing
Microsarcops cinereus (Blyth, 1842)	Grey-headed Lapwing
Charadrius a. alexandrinus L. 1758	Kentish Plover
C. dubius dubius Scopoli, 1786	Little Ringed Plover
C. dubius curonicus Gmelin, 1789	Ringed Plover
C. hiaticula L. 1758	Ringed Plover
Pluvialis pluvialis (L. 1758)	Golden Plover
Squatarola squatarola (L. 1758)	Grey Plover
Lobivanellus indicus indicus (Boddaert, 1783)	Goa Sandpiper
Lobibyx lobatus (Latham, 1801)	Wattled Sandpiper

Afribyx senegalus senegalus (L. 1766)	Senegal Sandpiper
Actitis hypoleucos (L. 1758)	Common Sandpiper
A. littoreus (L. 1766)	Ruff and Reeve
A. macularia macularia (L. 1766)	Spotted Sandpiper
SCOLOPACIDAE	
Numenius arquatus arquatus (L. 1758)	Curlew
N. phaeopus (L. 1758)	Whimbrel
N. tenuirostris Vieillot, 1817	Slender-billed Curlew
Limosa lapponica lapponica (L. 1758)	Bar-tailed Godwit
L. limosa limosa (L. 1758)	Black-tailed Godwit
Tringa flavipes (Gmelin, 1788)	Yellowshrank
T. ochropus L. 1758	Green Sandpiper
T. totanus (L. 1758)	Redshank
Capella g. gallinago (L. 1758)	Common Snipe
C. major (Gmelin, 1788)	Great Snipe
C. nigripennis (Bonaparte, 1839)	Ethiopian Snipe
Catoptrophorus s. semipalmatus (Gmelin, 1789)	Semipalmated Snipe
ARENARIINAE	
Arenaria interpres (L. 1758)	Turnstone
EROLIINAE	
Calidris canutus canutus (L. 1758)	Knot
Erolia alpina alpina (L. 1758)	Dunlin
E. testaceae (Pallas, 1764)	Curlew Sandpiper
Philomachus pugnax (L. 1758)	Ruff
RECURVIROSTRINAE	
Himantopus h. himantopus (L. 1758)	Black-winged Stilt
OTIDAE	
Chlamydotis u. undulatus (Jacquin, 1784)	Hubara or Ruffed Bustard
DROMADIDAE	
Burhinus oedicnemus oedicnemus (L. 1758)	Stone Curlew
Dromas ardeola Paykull, 1805	Crab Plover
GLAREOLIDAE	
Cursorius bicinctus Temminck, 1820	Double-banded Courser
LARIDAE	
Larus argentatus argentatus Pontoppidan, 1763	Herring Gull
L. ridibundus ridibundus L. 1766	Black-headed Gull
STERNIDAE	
Childonias nigra nigra (L. 1758)	Black Tern
STERCORARIDAE	
Stercorarius parasiticus (L. 1758)	Arctic Skua
Hydroprogne tschegrava tschegrava (Lepechin, 1770)	Caspian Tern
Thalasseus cantiaca Gmelin, 1788	Sandwich Tern
Sterna hirunda hirunda L. 1758	Common Tern
S. minuta L. 1766	Lesser Tern
Hydrochelidon leucoptera (Schinz, 1815)	White-winged Black Tern
RHYNCOPIDAE	
Rhynchops flavirostris Vieillot, 1816	Skimmer
R. nigra nigra L. 1758	Black Skimmer

ALCIDAE

Alca torda L. 1758	Razorbill
Cepphus grylle grylle (L. 1758)	Black Guillemot
Fratercula arctica arctica (L. 1758)	Puffin

DIOMEDEIDAE

Diomedea chlororhyncha Gmelin, 1788	Yellow-bellied Albatross
D. exulans L. 1766	Wandering Albatross
D. nigripes Audubon, 1839	Black-footed Albatross

PROCELLARIDAE

Puffinus assimilis baroli (Bonaparte, 1857)	Madeiran Little Shearwater
Daption capense (L. 1758)	Cape Petrel

COLYMBIDAE

Colymbus fluviatilis Tunstall, 1771	Diver
C. immer Brunnich, 1764	Diver
C. stellatus Pontoppidan, 1763	Diver
Urinator glacialis (L. 1766)	Great Northern Diver
U. septentrionalis L. 1766	Red-throated Diver
Podiceps cristatus cristatus (L. 1758)	Great Crested Grebe

RALLIDAE

Rallus a. aquaticus L. 1758	Water Rail
Hypotaenidia philippensis (L. 1766)	Phillipine Rail
Gallirallus a. australis (Sparrman, 1786)	South Island Weka Rail
Crex crex (L. 1758)	Corn Crake
Porzana fusca fusca (L. 1766)	Brown Rail
P. porzana (L. 1766)	Spotted Crake
P. pusilla pusilla (Pallas, 1776)	Dwarf Rail
Gallinula c. chloropus (L. 1758)	Moorhen
Porphyrio caeruleus (Vandelli, 1797)	Purple Waterhen

FULICINAE

Fulica atra atra L. 1758	Coot
F. cristata Gmelin, 1788	Crested Coot

HELIORNITHIDAE

Podica senegalensis (Vieillot, 1817)	Senegal Finfoot

COLUMBIDAE

Ducula goliath (Gray, 1859)	Fruit Pigeon
D. pinon pinon (Quoy & Gaimard, 1824)	New Guinea Imperial Pigeon
Lopophaps p. plumifera (Gould, 1842)	Bronze-winged Dove
Geotrygon a. albiefacies (Sclater, 1858)	White-faced Dove
Trugon terrestris terrestris Gray, 1849	Dove
Otidiphaps nobilis nobilis Gould, 1870	Dove
Caloenas n. nicobarica (L. 1758)	Nicobar Pigeon
Myrsticivora bicolor (Scopoli, 1786)	White Nutmeg Pigeon
Hemiphaga n. novaeselandiae (Gmelin, 1789)	New Zealand Pigeon
Columba domestica L. 1758	Domestic Pigeon
Ptilinopus pulchellus (Temminck, 1835)	Painted Pigeon
Megaloprepia magnifica puella (Lesson, 1827)	Purple-breasted Fruit Pigeon

GOURINAE

Goura coronata (L. 1758)	Crowned Pigeon
G. victoria (Fraser, 1844)	Victoria Crowned Pigeon

TETRAONIDAE	
Tetrao tetrix L. 1766	Black Grouse
T. urogallus urogallus L. 1758	Wood Grouse
PHASIANINAE	
Perdix perdix perdix (L. 1758)	Partridge
P. rufa (L. 1766)	Red-legged Partridge
P. saxatilis (Meyer, 1805)	Greek Partridge
Coturnix c. coturnix (L. 1758)	Quail
Rollulus roulroul (Scopoli, 1786)	Red-crested Wood Partridge
Tragopan satyra (L. 1758)	Crimson Tragopan
Gallus domesticus L. 1758	Domestic Fowl
Phasianus pictus L. 1766	Painted Pheasant
Ocydromus earlii Gray, 1862	Brown Wood Hen
NUMIDIDAE	
Numida m. meleagris (L. 1758)	Guinea Fowl
MELEAGRIDIDAE	
Meleagris g. gallopavo L. 1758	Mexican Turkey
MEGAPODIIDAE	
Megapodius freycineti freycineti Gaimard, 1823	New Guinea Quail
M. laperouse laperouse Gaimard, 1823	Marianne Island Quail
M. reinwardt forsteni Gray, 1847	Megapode
Talegalla cuvieri Lesson, 1828	Papuan Brush Turkey
Aepypodius bruijnii (Oustalet, 1880)	Brush Turkey
CRACIDAE	
Crax alector L. 1766	Guinea Crested Curassow
Ortalis arauchan squamata Lesson, 1829	Scaly Chachalaca
OPISTHOCOMIDAE	
Opisthocomus hoazin (Muller, 1776)	Hoatzin
GRUINAE	
Grus antigone antigone (L. 1758)	Sarus Crane
G. grus grus (L. 1758)	Common Crane
PSOPHIIDAE	
Psophia crepitans crepitans L. 1758	Grey-winged Trumpeter
TINAMIDAE	
Crypturus cinnamomeus (Lesson, 1842)	Cinnamon Tinamou
STRUTHIONIDAE	
Struthio camelus L. 1766	Ostrich
RHEIDAE	
Rhea americana (L. 1758)	Rhea

REFERENCES

BERLESE, A. (1882–98). Acari, Myriopoda et Scorpiones hucusque in Italia reperta, fasc. I–92.

BONNET, A. (1924). Revision des Genres Megninia, Mesalges et Genres voisins de la sous-famille des Sarcoptides Plumicoles. *Bull. Soc. zool. Fr.*, **49**, 146–88.

BONNET, A. & TIMON-DAVID, J. (1932). Sur quelques Oiseaux de Provence et leurs Acariens plumicoles. *Bull. Soc. linn. Provence*, **5**, 23–9.

BUCHHOLZ, R. (1869). *Bemerkungen über die Arten der Gattung* Dermaleichus Koch. Dresden.

CANESTRINI, G. (1878). Nuove specie del genere '*Dermaleichus*'. *Atti Ist. Veneto* (5), **5**, 1–68.

CANESTRINI, G. (1879). Intorno ad alcuni acari parassiti. *Atti Soc. Veneto-Trentina*, **6**, 7, 42.

CANESTRINI, G. (1881). *Atti Soc. Padau* (5), **7**, 1–28.

CANESTRINI, G. (1885–99). *Prospetto dell' Acarofauna Italiana.*

CANESTRINI, G. & BERLESE, A. (1880). *Atti Soc. Veneto-Trentina*, **7**, 145–7.

CANESTRINI, G. & BERLESE, A. (1881). *Atti Soc. Veneto-Trentina*, **8**, 145–53.

GERVAIS, P. (1844). *Acarides.* In Walckenaer's *Hist. Nat. Ins. Apt.* **3**, 262.

GIEBEL, C. J. (1871). Ueber die Federmilbengattung *Analges* (Nitzsch). *Z. Naturw.* **37**, 490–8.

GMELIN, J. F. (1788). In Linné, *Syst. Nat.* (ed. 13), **5**, 2930.

HALLER, G. (1877). Revision der Gattung *Analges* sive *Dermaleichus* Koch. *Z. wiss. Zool.* **30**, 50–81.

HALLER, G. (1878). Weitere Beiträge zur Kenntniss der Dermaleichen Koch. *Z. wiss. Zool.* **30**, 511–62.

HALLER, G. (1882*a*). Zur Kenntniss der Dermaleichiden. *Arch. Naturgesch.* **48**, 47–79.

HALLER, G. (1882*b*). Über den Bau der Vogelbewohnenden Sarcoptiden (Dermaleichidae). *Z. wiss. Zool.* **36**, 377.

HERMANN, J. F. (1804). *Mémoire Aptérologique.* Strasbourg.

HULL, J. E. (1931). A new genus and species of Analgesidae (Feather Mites). *Vasculum*, **17**, 145–7.

HULL, J. E. (1934). Concerning British Analgesidae (Feather Mites). *Trans. North. Nat. Union*, **1**, 200–6.

KOCH, C. L. (1835–44). *Crustaceen, Myriapoden und Arachniden Deutschlands.* Regensburg.

KRAMER, P. (1899). See Canestrini, G. & Kramer, P., 'Demodicidae und Sarcoptidae', *Das Tierreich*, **7**, 1–193.

LATREILLE, P. A. (1796). *Précis des Caractères génériques des Insectes, disposés dans un Ordre naturel.* Paris.

LATREILLE, P. A. (1806–9). *Genera Crustaceorum et Insectorum secundum Ordinem naturalem in Familias disposita, Iconibus Exemplisque plurimis explicata.*

LINNÉ, C. von (1758). *Systemae Naturae* (ed. 10), **1**, 616.

MEGNIN, P. (1877). See Robin, C. & Megnin, P.

MEGNIN, P. & TROUESSART, E. L. (1844). *J. Microg.* **8**, 150–434.

MICHAEL, A. D. (1881). *J. R. Micr. Soc.* (2), **1**, 212–16.

NEUMANN, G. (1887). See Trouessart, E. L. & Neumann, G.

NITZSCH, C. L. (1818). *Acarina* in Ersch & Gruber, *Allg. Encycl. Wiss.* **1**, 246–52.

NORNER, C. (1882). *Analges minor*, eine neue Milbe im Innern der Federspulen der Huhner. *Ver. Ges. Wien*, **32**, 387.

NORNER, C. (1883). Beitrag zur Kenntnis der Milbenfamilie der Dermaleichiden. *Ver. Ges. Wien*, **33**, 91–106.

OUDEMANS, A. C. (1898). List of Dutch Acari, Seventh Part. *Tijdschr. Ent.* **40**, 250–69.

OUDEMANS, A. C. (1904). *Acarologische Aanteekeningen.* 13. *Ent. Ber., Amst.*, **1**, 169–74.

OUDEMANS, A. C. (1905*a*). *Acarologische Aanteekeningen.* 15. *Ent. Ber., Amst.*, **1**, 207–10.

OUDEMANS, A. C. (1905*b*). *Acarologische Aanteekeningen.* 16. *Ent. Ber., Amst.*, **1**, 216–18.

OUDEMANS, A. C. (1905*c*). *Acarologische Aanteekeningen.* 17. *Ent. Ber., Amst.*, **1**, 222–6.

OUDEMANS, A. C. (1905*d*). *Acarologische Aanteekeningen.* 18. *Ent. Ber., Amst.*, **1**, 236–41.

RIVOLTA, S. (1876). Nuova forma di rogna nei polli. *Giornale di Anatomia. Fisiologia e Patologia degli Animali*, **8**, 1–247. Pisa.

ROBIN, C. (1868). Mémoire sur les Sarcoptides avicoles et sur les métamorphoses des acariens. *C.R. Acad. Sci., Paris*, **66**, 776–86.

ROBIN, C. (1877). See Robin, C. & Megnin, P.

ROBIN, C. & MEGNIN, P. (1877). Mémoire sur les Sarcoptides plumicoles. *J. Anat. Phys.* **13**, 209–48, 391–429, 498–519, 629–56.

SUGIMOTO, M. (1940). Studies on the Formosan mites (3rd Report). On the feather mites, Analgesidae Canestrini, 1892. *Bull. Sch. Agric. Forest. Taihoku. Imp. Univ.* **1**, 40–58.

TROUESSART, E. L. (1884*a*). Sur les Acariens qui vivent dans le tuyau des plumes des oiseaux. *C.R. Acad. Sci., Paris*, **94**, 1130–3.

TROUESSART, E. L. (1884*b*). Les Sarcoptids Plumicoles. *J. Microg.* **8**, 527–32, 572–9.

TROUESSART, E. L. (1885*a*). Les Sarcoptides plumicoles ou Analgesines. I. *Les Ptéroliches. J. Microg.* **9**, 63–117.

TROUESSART, E. L. (1885*b*). Notes sur la classification des Analgesides, et diagnoses d'espèces et de genres nouveaux. *Bull. Soc. Angers*, **14**, 46–89.

TROUESSART, E. L. (1885*c*). Les Acariens parasites des Calaos: *l'Oustaletia pegasus. La Nature*, 1885 (2), pp. 24–6.

TROUESSART, E. L. (1886*a*). Diagnoses d'espèces nouvelles de Sarcoptides plumicoles. *Bull. Soc. Angers*, **16**, 85–156.

TROUESSART, E. L. (1886*b*). Monographie du genre *Freyana* (Haller) et description des espèces nouvelles du Musée d'Angers. *Bull. Soc. Angers*, **16**, 95–155.

TROUESSART, E. L. (1892). Considérations générals sur la classifications des Acariens. *Rev. Sci. nat. Ouest, Paris*, **2**, 50, 20–54.

TROUESSART, E. L. (1895*a*). Sur le genre *Analges* (Sarcoptidae) et remarques critiques sur les espèces récemment décrites par M. Hugo Zimmermann. *Ann. Soc. ent. Fr.* **63**, ccxliii–ccxlviii.

TROUESSART, E. L. (1895*b*). Description de trois nouvelles espèces de grande taille du groupe des Sarcoptides plumicoles. *Ann. Soc. ent. Fr.* **64**, cccxi–cccxiii.

TROUESSART, E. L. (1896). Sur la classification des Sarcoptides plumicoles. *Acariens. Bull. Soc. ent. Fr.* **20**, 419–22.

TROUESSART, E. L. (1897*a*). Application de photographie microscopique à l'étude des Sarcoptides plumicoles. *Bull. Mus. Paris*, **3**, 98–101.

TROUESSART, E. L. (1897*b*). Sur la classification des Sarcoptides plumicoles (Analgesidae), nouvelle note. *Bull. Soc. ent. Fr.* **1896**, 419–22.

TROUESSART, E. L. (1898*a*). Diagnoses préliminaires d'espèces nouvelles de Sarcoptides. *Bull. Soc. ent. Fr.* **1898**, 289–92, 309–11, 319–22.

TROUESSART, E. L. (1898*b*). Sur un nouveau genre de Sarcoptides plumicoles. *C.R. Soc. Biol., Paris*, **5**, 970–2.

TROUESSART, E. L. (1899). Diagnoses préliminaires d'espèces nouvelles d'Acariens plumicoles. Additions et corrections à la sous-famille des Analgesines. *Bull. Soc. Angers*, **28**, 21–176.

TROUESSART, E. L. (1915). Revision des genres de la sous-famille des Analgesinae ou Sarcoptides plumicoles. *Bull. Soc. Zool. Fr.* **40**, 207–23.

TROUESSART, E. L. (1919*a*). Diagnose de genres nouveaux de Sarcoptides plumicoles. *Ann. Mag. nat. Hist.* **4**, 336–8.

TROUESSART, E. L. (1919*b*). Monographie de genres *Hemialges* et *Hyperalges* (Sarcoptides plumicoles) *Bull. Soc. zool. Paris*, **44**, 302–21.

TROUESSART, E. L. & MEGNIN, P. (1884). Diagnoses des Espèces et Genre nouveaux de Sarcoptides Plumicoles *Le Naturaliste*, **2**, no. 50, 403–5.

TROUESSART, E. L. & NEUMANN, G. (1887). Types nouveaux de Sarcoptides épidermicoles et psoriques. *Bull. Soc. Angers*, **17**, 121–54.

TROUESSART, E. L. & NEUMANN, G. (1888). Diagnoses d'espèces nouvelles de Sarcoptides plumicoles. *Bull. Sci. Fr. Belg.* **19**, 333–71.

TRYYELL, J. B. (1882). On some Canadian ectoparasitic Sarcoptidae. *Ottawa Fld Nat. Cl.* **3**, 43–8.

VITZTHUM, H. GRAF (1922). *Acarologische Beobachtungen*, 6. Reihe. Die Gattung *Proctophyllodes* Robin, 1868. *Arch. Naturgesch.* **88**, 68–85.

VITZTHUM, H. GRAF (1934). *Microlichus uncus* n.sp. *Bull. Mus. Hist. nat. Belg.* **10**, 12, 1–20.

(*MS. received for publication* 9. x. 1951.—Ed.)

FIVE NEW SPECIES OF LARVAL MITES (ACARINA: TROMBICULINAE)

By CHARLES D. RADFORD

Membre Correspondant, Muséum National d'Histoire Naturelle, Paris

(With 10 Figures in the Text)

INTRODUCTION

Prior to 1939 our knowledge of the mites of the subfamily Trombiculinae was limited to about a dozen species, whose life history had been the subject of investigations by Nagayo and his associates in Japan, Walch in the Dutch East Indies and Gater, Fletcher, Lesslar and Lewthwaite in Malaya.

In India a number of cases of scrub-typhus were recorded among military personnel in the early nineteen-thirties, and between 1935 and 1939 Covell and his colleagues carried out a series of researches on the epidemiology of the disease in the Simla Hills.

During the second World War, outbreaks occurred from time to time among troops operating in Ceylon, Burma and in various islands in the South-West Pacific. These outbreaks threatened to become a serious drain on manpower, and a number of investigations were set on foot with a view to bringing them under effective control.

These wartime investigations brought to light many new species of larval Trombiculinae, and a fair amount of knowledge was gained in the technique of breeding them and studying their adult and nymphal forms which are free living, whereas the larvae are parasitic on mammals, birds, reptiles and man.

Since the war many fresh research projects have been conducted in Malaya, the U.S.A. and other countries, in consequence of which this very large and interesting subfamily of mites has become one of the most widely studied groups in acarology.

This paper is offered as a small contribution to our knowledge of the larvae of these mites. Five new species are herein described and figured. They differ from all other known species in the shape of the dorsal scutum and the shape and number of spines.

In recognition of his researches on scrub-typhus in the Simla Hills, I name one of these species *Neoschöngastia covelli* for Major-General Sir Gordon Covell, Kt., C.I.E., and one *Trombicula kalrai* in recognition of the valuable assistance given to me during the wartime research, and the collections of mites which were subsequently sent to me by my former colleague Major S. L. Kalra, I.A.M.C.

The type specimens are retained in the author's private collection.

Genus **Trombicula** Berlese, 1905

Trombicula kalrai n.sp.

The larval dorsum (Fig. 1) shows this species to differ from all the known species of this genus in the exceptionally large number of dorsal and ventral spines and their extreme length.

The dorsal scutum is wider than long, broadly rectangular in shape, with its posterior edge slightly rounded, and with the lateral edges concave except for an expansion round the base of the postero-lateral spines. In the single specimen the anterior median spine is missing, also the sensory spines. Level with and flanking the postero-lateral spines is the anterior eye which is almost twice the size of the posterior eye. The dorsal spines are so numerous and closely situated that there would appear to be no recognizable rows as is usual in the Trombicula. There are about 112 spines on the dorsum and two pairs of humeral spines. All legs are composed of seven segments; all tarsi are provided with a pair of claws and a long, slender empodium. There are no mastitarsala on tarsus iii.

On the venter (Fig. 2) the spines are seen to approximate closely to those of the dorsum both in their length and stoutness. One pair of spines is placed between coxae i, and three pairs lie between coxae iii. Posterior to coxae iii there are approximately ninety spines. Each coxa has a single spine.

The palpal claw is tridentate. First palpal spine nude, also the 2nd, 3rd and 4th. The chelicera has a tri-cuspid apical tooth. Galeal seta provided with lateral barbs.

Standard data. AW 60 μ, PW 79 μ, SB 28 μ, ASB 28 μ, PSB 12 μ, A–P 40 μ, AM ?, AL 44 μ, PL 54 μ, Sens ?, DS 54 μ, SD 52 μ.

Host. A rat (*Rattus* sp.)

Locality. Kumoan Hills, India. October 1946. S. L. Kalra.

Trombicula plumosa n.sp.

The dorsum of the larva (Fig. 3) shows the species to differ from all others of this genus in the shape of the spines, which are widely expanded, plumose.

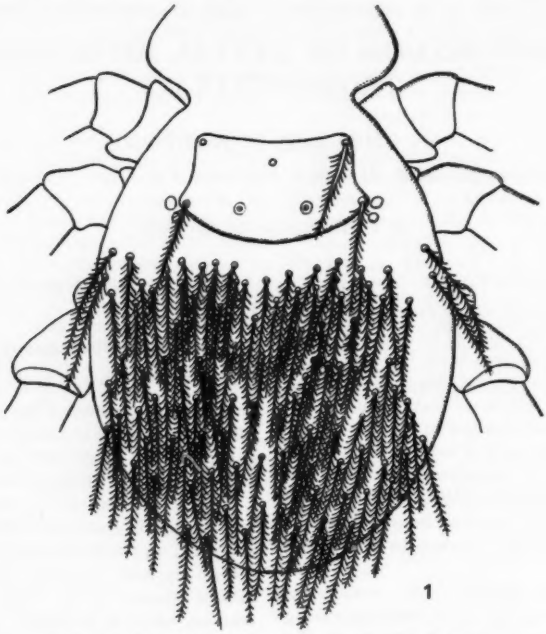


Fig. 1. *Trombicula kalrai* n.sp. Dorsum of larva.

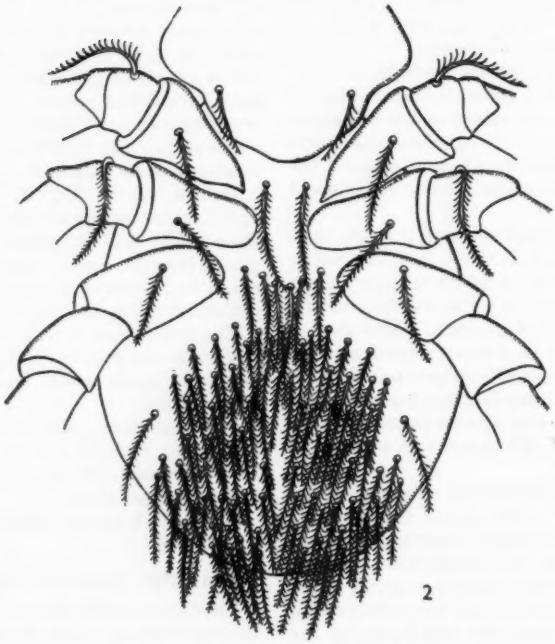


Fig. 2. *T. kalrai* n.sp. Venter of larva.

This species differs from *T. intermedia* Nagayo, 1920, in the possession of a greater number of dorsal spines.

The dorsal scutum is rectangular in shape, with its anterior edge slightly concave, the lateral edge is almost straight, and the posterior edge is slightly rounded except for a median concavity. The antero-lateral spines are simple, unexpanded; the antero-median spine is plumose, its base placed some distance back from the anterior edge of the scutum; arranged in rows of 4, 4, 10 and 6. Posterior to these there are some thirty-four stout, expanded spines which are arranged as in the figure.

The palpal claw is tridentate. First palpal spine nude; 2nd spine is long, nude, its length being equal to the second and third palpal segment; 3rd spine nude; 4th spine with lateral barbs. Chelicera with a single tooth distally. Galeal seta with seven lateral barbs.

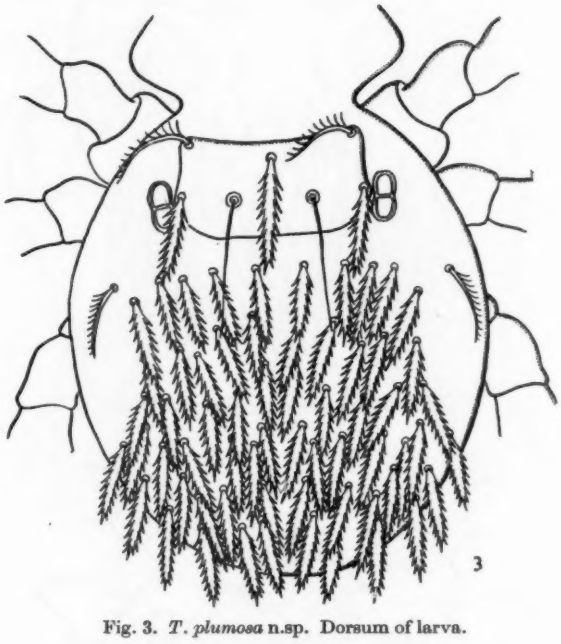


Fig. 3. *T. plumosa* n.sp. Dorsum of larva.

the postero-lateral spines are plumose, their bases being just anterior of the postero-lateral angles of the scutum, and anterior to the bases of the sensory spines. The sensory spines are long, whip-like and with a few lateral barbs in their distal third. The sensory spine bases are equidistant from each other and from the postero-lateral spines. The humeral spines are simple. On the dorsum there are about sixty spines arranged in uneven rows of 10, 10, 2, 8, 2, 10, 4, 8 and 6.

Eye plates are present on each side of the dorsal scutum, level with the postero-lateral spines; the paired eyes on each plate being equal in size. All legs with seven segments; all tarsi are provided with a pair of claws and a simple empodium.

The venter of the larva (Fig. 4) shows a pair of long spines between coxae i, with a pair of shorter spines between coxae iii. Posterior to coxae iii there are four rows of short, simple spines, which are

Standard data. AW 70 μ, PW 76 μ, SB 33 μ, ASB 27 μ, PSB 13 μ, A–P 26 μ, AM 40 μ, AL 40 μ, PL 43 μ, Sens 59 μ, DS 40 μ, SD 40 μ.

Host. A squirrel (*Callosciurus pygerythrus pygerythrus* Geoffroy).

Locality. Imphal, Manipur State, India. 14 February 1946. J. Hake.

Note. In *Trombicula intermedia* Nagayo, 1920 all the ventral spines are plumose.

Genus **Neoschöngastia** Ewing, 1929

Neoschöngastia covelli n.sp.

This species differs from the others of the genus in the width of the dorsal scutum, the large balloon-like sensory spines, the expanded antero-median spine, and the length of the postero-lateral spines.

The dorsum of the larva (Fig. 5) shows the scutum to be wider than long, its anterior edge

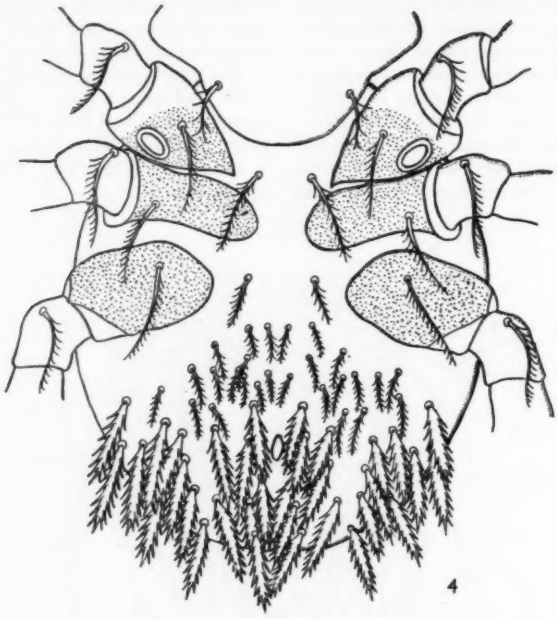


Fig. 4. *T. plumosa* n.sp. Venter of larva.

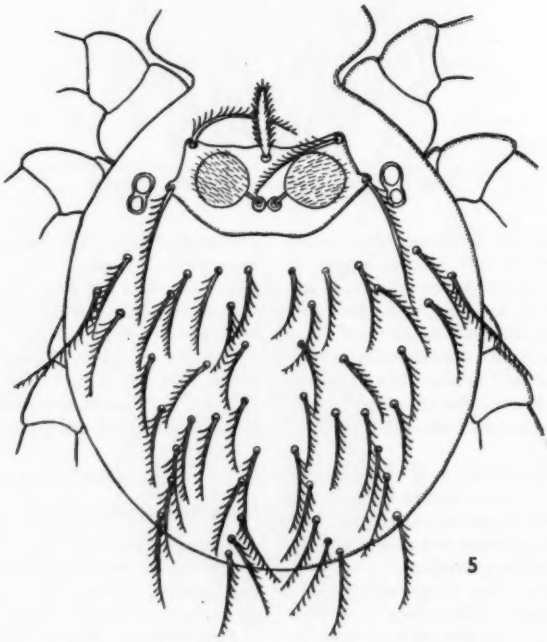


Fig. 5. *Neoschöngastia covelli* n.sp. Dorsum of larva.

having a small concavity on each side of the antero-median spine; the lateral edge is almost straight with a slight bulge at the posterior angles; the posterior edge is rounded. The antero-median spine is broad, spatulate and is not placed on the anterior edge of the scutum; the antero-lateral spines are long and simple; the postero-lateral spines are of exceptional length and placed on lateral projections of the scutum. The sensory spines are balloon-like, capitate, with short hairs over their surface, and are than the others. On coxae i and ii there is a single spine; on coxa iii there are two spines, both close to the anterior edge of the coxa.

The palpal claw is tridentate. First and 2nd palpal spine with lateral barbs; the 3rd, 4th and 5th palpal spines are nude. Chelicera with a single tooth near its apical end. Galeal seta is nude.

Standard data. AW 63 μ, PW 80 μ, SB 13 μ, ASB 27 μ, PSB 16 μ, A–P 20 μ, AM 33 μ, AL 50 μ, PL 55 μ, Sens 33 μ, DS 40 μ, Humerals 53 μ, SD 43 μ.

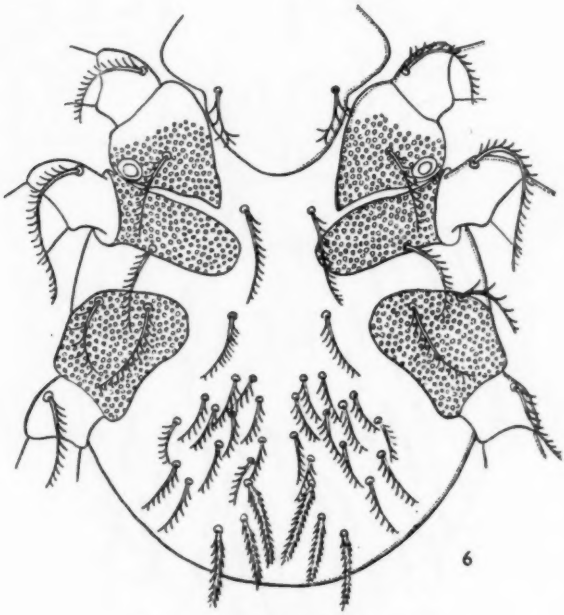


Fig. 6. *N. covelli* n.sp. Venter of larva.

borne upon short stalks. The sensory spine bases are placed close together and lie posterior to a line drawn between the postero-lateral spine bases. On the dorsum there are about forty-six spines arranged in transverse rows of 4, 10, 2, 6, 8, 4, 4, 4 and 4. In length these dorsal spines are about the length of the antero-lateral spines. The lateral or humeral pair of spines in row one are almost as long as the postero-lateral spines.

Eye plates are present on each side of the dorsal scutum, level with the postero-lateral spines; the anterior eye being almost twice the size of the posterior eye. All legs with seven segments; all tarsi with a pair of claws and a simple empodium.

On the venter (Fig. 6) there are three pairs of expanded, spatulate spines at the posterior end of the body, all the other ventral spines are simple. The pair of spines lying between coxae i are longer

Host. A squirrel (*Callosciurus pygerythrus pygerythrus* Geoffroy).

Locality. Imphal, Manipur State, India. 14 February 1946. J. Hake.

Genus **Ascoschöngastia** Ewing, 1946

Ascoschöngastia capillata n.sp.

The dorsum of the larva (Fig. 7) shows this species to have a squat, rectangular scutum, with the postero-lateral spines clear of the posterior angles of the scutum, the striations of the dorsum being distinct between the scutum and these spines. The scutum is only slightly wider than deep; the anterior edge of the scutum has a slight concavity on each side of the antero-median spine; the lateral edge has a median concavity; the posterior edge is concave medially. The three anterior spines are of

almost equal length and placed along the anterior edge of the scutum; the postero-lateral spines are longer than the others. The sensory spines are elongated, club-shaped, and covered with small spines. Anterior to the sensory spine bases there is a ridge. Flanking the dorsal scutum and anterior to the sensory spines are the eye-plates; the anterior eye being larger than the posterior. On the dorsum there are some seventy-eight spines, arranged in rows of 4 (humerals), 12, 4, 10, 12, 12, 8, 8, 4 and 4.

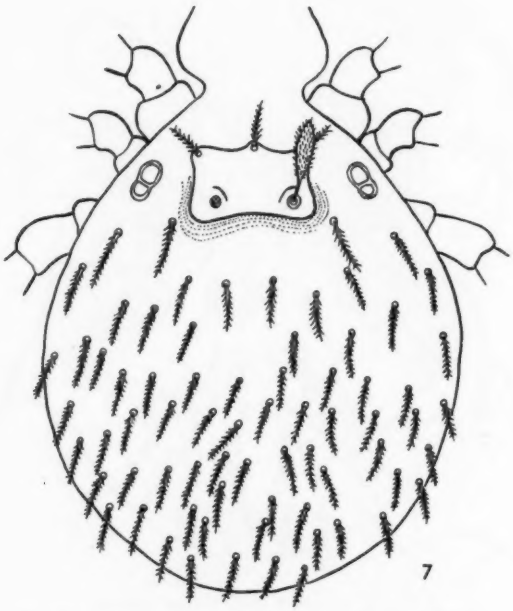


Fig. 7. *Ascoschöngastia capillata* n.sp. Dorsum of larva.

All legs composed of seven segments; all tarsi with a pair of claws and a simple empodium. No mastitarsala on the third tarsus.

On the venter of the larva (Fig. 8) there are about sixty short, simple spines posterior to the third pair of legs. Each coxa has a single spine.

The palpal claw is tridentate. The 1st and 2nd palpal spines are barbed; 3rd, 4th and 5th palpal spines nude. Chelicera with an apical tooth. Galeal seta nude.

Standard data. AW 56 μ, PW 60 μ, SB 30 μ, ASB 22 μ, PSB 8 μ, A–P 30 μ, AM 20 μ, AL 20 μ, PL 30 μ, Sens 42 μ, DS 20 μ, Humerals 30 μ, SD 28 μ.

Host. A rat (*Rattus* sp.).

Locality. Jubbulpore, Central Provinces, India. April 1947. S. L. Kalra.

Genus **Comatacarus** Ewing, 1942

Comatacarus atollensis n.sp.

The dorsum of the larva (Fig. 9) shows the scutum to be broader than long, roughly pentagonal; its anterior edge with concavities between the antero-median and the antero-lateral spines; lateral edge is concaved medially; the posterior edge extends to a blunt point. The antero-lateral spines are placed some distance from the antero-lateral angles of the scutum; the paired antero-median spines are close together and anterior to a line drawn between the antero-lateral spines; the postero-lateral spines are posterior to the level of the sensory spine bases. The sensory spines are long, simple, with lateral barbs on the distal two-thirds of their length. The sensory spine bases are about equidistant from one another and the lateral edge of the scutum. Flanking the scutum are the eye-plates, level with the postero-lateral spines. The paired eyes are of equal size. All legs with seven segments; all tarsi provided with a pair of claws and a simple empodium. On tarsus three there is a single mastitarsala.

On the dorsum there are some twenty-six spines arranged in rows of 2, 6, 6, 6, 4 and 2.

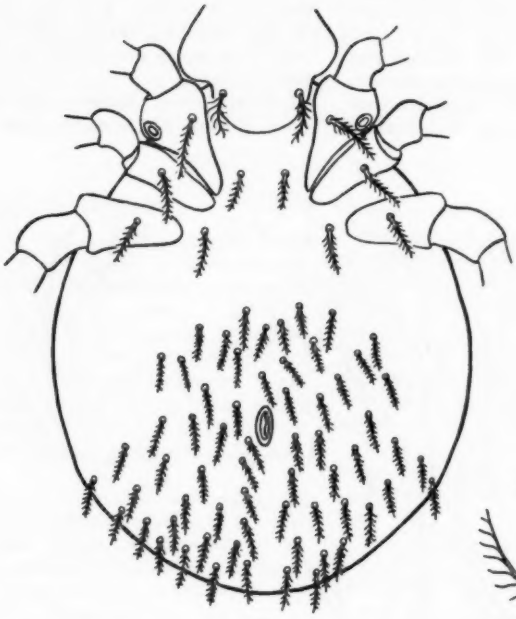


Fig. 8. *A. capillata* n.sp. Venter of larva.

Fig. 8*a*. *A. capillata* n.sp. Palp of larva.

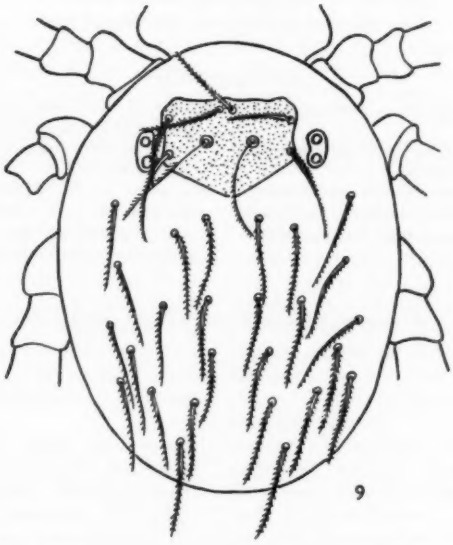


Fig. 9. *Comatacarus atollensis* n.sp. Dorsum of larva.

On the venter of the larva (Fig. 10) there are few spines, which are arranged as in the figure. Each coxa has a single spine.

The palpal claw is tridentate. The 1st and 2nd palpal spine is provided with lateral barbs; the 3rd, 4th and 5th palpal spines are nude. Chelicera with two teeth at the distal end as figured. Galeal seta?

Standard data. AW 55 μ, PW 55 μ, SB 18 μ, ASB 22 μ, PSB 23 μ, A–P 15 μ, AM 35 μ, AL 30 μ, PL 45 μ, Sens 17 μ, DS 40 μ, SD 45 μ.

Host. A brown rat (*Rattus norvegicus norvegicus* Berkenhout).

Locality. Gan, Maldive Islands, Addu Atoll, Indian Ocean. 27 December 1944. C. D. Radford.

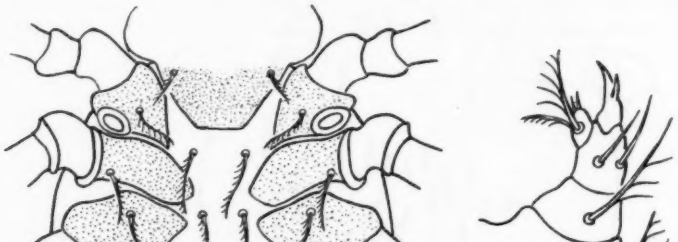


Fig. 10. *C. atollensis* n.sp. Venter of larva.
Fig. 10*a*. *C. atollensis* n.sp. Palp of larva.
Fig. 10*b*. *C. atollensis* n.sp. Chelicera of larva.

REFERENCES

BERLESE, A. (1905). Acari Nuovi. Manipulus IV. *Redia*, **2**, 154–76.

EWING, H. E. (1929). *A Manual of External Parasites*, pp. xiv + 225. Springfield, Illinois.

EWING, H. E. (1942). Remarks on the taxonomy of some American chiggers (Trombiculinae) including the descriptions of new genera and species. *J. Parasit.* **28**, 485–93.

EWING, H. E. (1946). Notes on the taxonomy of three genera of trombiculid mites (chigger mites), together with the description of a new genus. *Proc. Biol. Soc. Wash.* **59**, 69–72.

WHARTON, G. W., JENKINS, D. W., BRENNAN, J. M., FULLER, H. S., KOHLS, G. M. & PHILIP, C. B. (1951). The terminology and classification of Trombiculid mites (Acarina: Trombiculidae). *J. Parasit.* **37**, 13–31.

(*MS. received for publication* 7. XII. 1951.—Ed.)

FOUR NEW SPECIES OF PARASITIC MITES (ACARINA)

By CHARLES D. RADFORD, *Membre Correspondant, Muséum National d'Histoire Naturelle, Paris*

(With 11 Figures in the Text)

INTRODUCTION

In this paper four new mites are described and figured, one being a new species of the subfamily Macronyssidae, one in the subfamily Laelaptinae and two in the family Trombiculidae.

Hirst (1921) described the new species *Liponyssus sternalis* which had been taken on an undetermined species of bat from Lake Beslik, Salonica. In the anterior angles of the sternal scutum of the female mite there was a window-like pore, in addition to the customary three pairs of sternal setae, and two pairs of lyriform pores.

Fonseca (1948), in his monographic work on the Macronyssidae (Liponyssidae), established the new genus *Hirstesia* with *Liponyssus sternalis* Hirst, 1921, as the genotype for those mites 'whose sternal plate shows two organs of unknown function'.

The author (1951) described the new species *Hirstesia kenyaensis* for another mite parasitic on an unidentified bat taken at Ngong, Kenya. In the species described herein the scale-like window or pore in the anterior angles of the sternal scutum of the female differs markedly from that of the other species *sternalis* and *kenyaensis*. To this new species I have given the name *Hirstesia britteni* in recognition of the eminent entomologist Harry Britten, A.L.S., F.R.E.S., of Manchester. He has been a staunch friend and has supplied me with many interesting specimens over the past twenty years. To him I owe a deep debt of gratitude for guiding my scientific studies in the early days.

The Dermapteron (*Hemimerus talpoides* Walker, 1909) is found as a parasite on the giant rat (*Cricetomys gambianus* Waterhouse, 1840). W. D. Hincks, Entomologist, Manchester Museum, passed on to me some mites that had been sent to him which Dr W. Peters had found on the *Hemimerus*. The mites are obviously proper to the subfamily Laelaptinae and, as there is no genus into which they could be appropriately placed, it seems desirable to establish a new genus.

I accordingly name these mites *Andréacarus petersi* n.g., n.sp. in honour of my esteemed friend and co-worker Dr Marc André, Sous-Directeur, Muséum National d'Histoire Naturelle, Paris, who has done so much to add to our knowledge of the Acarina, and in recognition of the donor Dr W. Peters.

The other two new mites are species of larval Trombiculinae, one being a new species of the genus *Acomatacarus* of the subfamily Leeuwenhoekiinae, the other in the genus *Trombicula*.

The mite *Acomatacarus keegani* n.sp. I have named in honour of my friend Capt. Hugh L. Keegan, Medical Service Corps, U.S. Air Force, whose contributions to Acarology are well known. The mite *Trombicula samara* n.sp. was also found on the same host by Keegan.

To all of the above gentlemen I wish to acknowledge my thanks for having given me the opportunity to examine the material and describe them.

Genus **Hirstesia** Fonseca, 1948

Hirstesia britteni n.sp.

The female venter (Fig. 1) shows the sternal scutum to be wider than long and lying between coxae ii, its anterior edge almost straight; its lateral edges are concave; the posterior edge is deeply arched. The sternal scutum bears three pairs of spines and two pairs of lyriform pores and, in its anterior angles, there are window-like, oval pores which differ from those found in the other species of this genus. The sternal spines are hollow (Fig. 2). Anterior to the sternal scutum there is a weakly chitinized jugularia with scale-like markings. The metapodal platelet is vestigial but the metapodal spine and third lyriform pore are present, level with coxa iii. The genital pore is covered by an apron which is ornamented with scale-like markings.

The genito-ventral scutum is tongue-shaped and bears, in addition to the pair of genital spines level with coxa iv, three other spines, one pair laterally about midway down the scutum, and a spine at the posterior tip. The anterior part of the scutum is weakly chitinized and its outline cannot be seen distinctly. In this genus there are normally three pairs of spines on the genito-ventral scutum, but in the present species the third pair of spines is placed just off the scutum. The anal scutum is widely separated from the genito-ventral scutum, and in shape resembles an inverted flask with an elongated neck. The anal pore lies well forward on the scutum, the paired anal spines lie on a level with the middle of the pore, the unpaired spine is situated about its own length from the posterior tip of the scutum.

Surrounding the genital and anal scutum on the soft integument of the venter there are some forty-eight pairs of spines.

Legs ii stouter and shorter than the others: on the anterior dorsal edge of coxa ii there is a cone-shaped tooth; on the posterior edge of coxa iv ventrally there is also a tooth-like process. Chaetotaxy of the legs not unlike other species of the Macronyssidae, presenting no unusual features.

The dorsal scutum is shown in dotted outline and it will be seen that, from the level of the third pair of legs the scutum leaves a gradually widening margin of the dorsal surface exposed until it is posterior to the fourth pair of legs from whence it is rapidly constricted to form a medially truncated lobe in the region of the anal scutum. Spines of the dorsal scutum long, slender, the two spines at the posterior edge of the scutum being longer and slightly stouter.

Host. A pipistrelle bat (*Pipistrellus pipistrellus* Shreber, 1774).

Locality. Bowdon, Altrincham, Cheshire. J. Armitage. 12 August 1951.

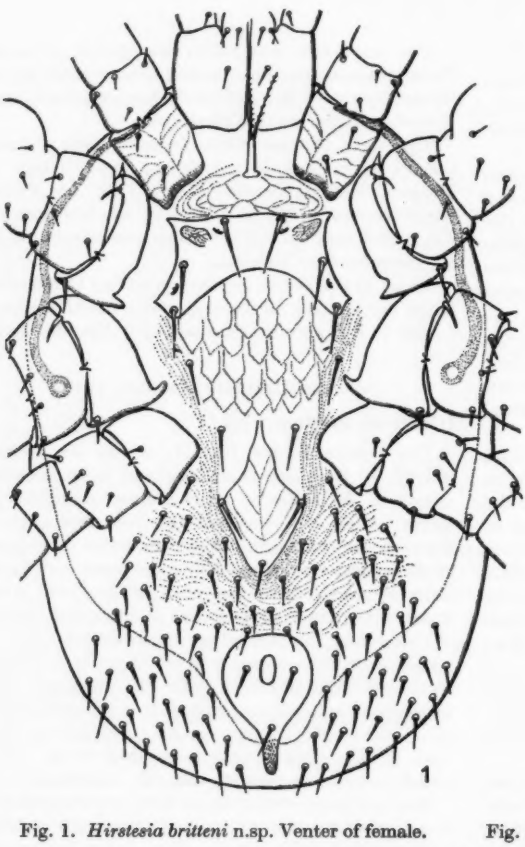


Fig. 1. *Hirstesia britteni* n.sp. Venter of female.

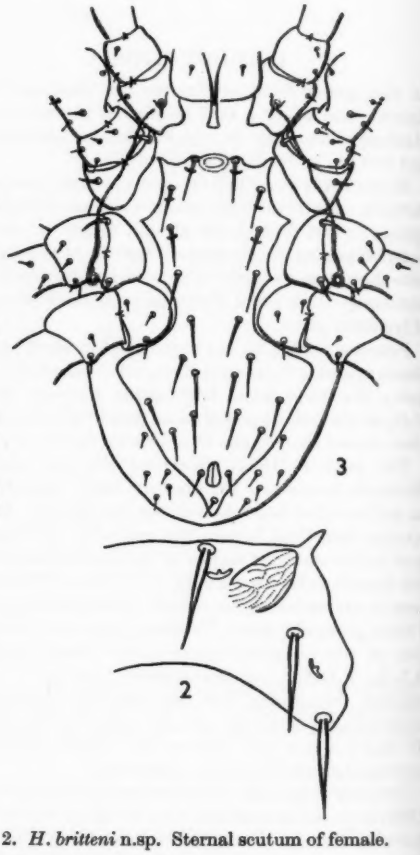


Fig. 2. *H. britteni* n.sp. Sternal scutum of female.

Fig. 3. *Andréacarus petersi* n.g., n.sp. Venter of male.

Measurements. ♀, 0·55 × 0·35 mm. (without capitulum). The male is unknown.

Genus **Andréacarus** n.g.

Male. All ventral scutae united into a holoventral scutum and bearing, in addition to the genital pore on the anterior edge, ten pairs of spines in addition to the customary three anal spines and the three pairs of lyriform pores. Holoventral scutum placed some distance from the tritosternum, its anterior

edge below the level of the anterior edge of coxa ii; laterally with concavities round coxae ii, iii and iv; scutum expanded behind coxa iv, from whence it tapers to a blunt point behind the anal pore. Only five pairs of small spines are present on the exposed integument of the venter. On the ventral surface of coxa i there is a stout spine. The dorsal scutum is entire, covering most of the body but leaving a narrow border of the dorsum exposed, round its posterior edge.

Female. Closely similar to the mites of the genus *Laelaps* Koch, 1835, but differing from all others in the subfamily Laelaptinae by the fusion of the anal scutum to the genito-ventral scutum.

The sternal scutum is broadly oblong, with two pairs of lyriform pores and three pairs of spines; metapodal platelets present, each bearing a single spine. The genito-ventral scutum is ewer-shaped and bears four pairs of spines as in the females of the genus *Laelaps*. Anal scutum broadly rounded anteriorly and fused with the posterior edge of the genito-ventral scutum; the anal pore lying well back on the scutum. Coxa i bearing a stout spine on the ventral surface as in the male. The dorsal scutum entire, but not covering the body, leaving a wide margin of integument exposed.

Material. Males and females found on a Dermapteron (*Hemimerus talpoides* Walker, 1909).

Genotype. Andréacarus petersi n.g., n.sp.

Andréacarus petersi n.sp.

The male venter (Fig. 3) shows the holoventral scutum to cover most of the body from coxa ii to behind coxa iv where it is expanded. From here it is tapered to a blunt point behind the anal pore. On the anterior edge of the scutum the genital pore is borne medially, this being flanked by a concavity on each side; the anterior angles of the scutum are not extended to between coxae i and ii, but some distance from the anterior edge of coxa ii; the lateral edge of the scutum is concave in the regions of coxae ii, iii and iv, and is expanded behind coxa iv; from here it tapers to a blunt point behind the anal pore, leaving a wide area of the body exposed. Flanking the genital pore on each side is the first sternal spine and lyriform pore; sternal spines ii and iii with the second and third lyriform pores are placed farther back on the side of the scutum. Level with the division between coxae iii and iv is the metapodal spine, and the genital spines are level with the middle of coxa iv. Posterior to coxa iv there are five pairs of long spines on the scutum, plus the three anal spines. Flanking the posterior end of the scutum there are five pairs of small spines.

The second pair of legs is stouter and shorter than the others; on the ventral surface of coxa i there is a stout spine or spur. Chaetotaxy of the legs normal for the Laelaptinae. The stigmal pore lies on a level with coxa iii and the peritreme extends anteriorly to the middle of coxa i.

The dorsal scutum does not completely cover the body leaving a margin of exposed integument from about the middle of legs iii to the posterior end of the body. Dorsal spines moderate except the pair at the terminal end of the scutum, which are longer and stouter.

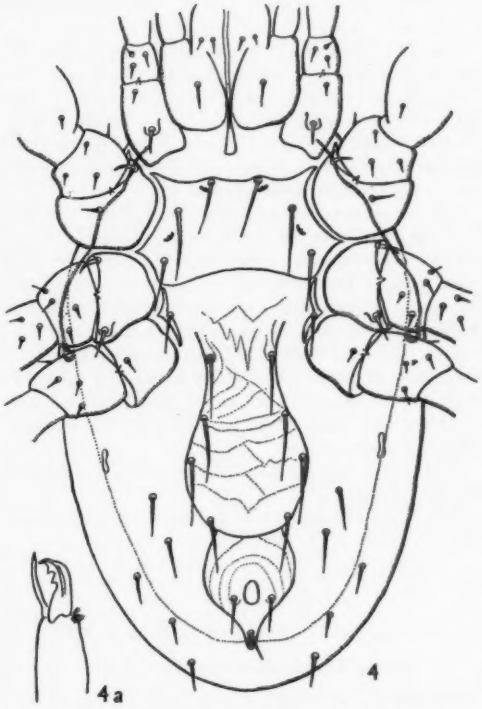


Fig. 4. *A. petersi* n.g., n.sp. Venter of female.
Fig. 4*a*. *A. petersi* n.g., n.sp. Chelicera of female.

The female venter (Fig. 4) shows the sternal scutum to be broadly oblong; its anterior edge has three concavities; the anterior angles of the scutum extend to the space between coxae i and ii; the lateral edge is concave to accommodate coxa ii; the posterior angle is extended between coxae ii and iii; the sternal scutum is extended posteriorly to about the middle of coxa iii; the posterior edge of the scutum is slightly concave. In addition to the customary three pairs of spines there are two pairs of lyriform pores. Level with the division of coxae ii and iv are the metapodal platelets on each of which is the metapodal spine.

The genito-ventral scutum is ewer-shaped with a rounded posterior edge and bears four pairs of spines; the genital spines are level with the middle

of coxa iv, the other three pairs of spines are borne on the lateral edge of the scutum. The anal scutum is fused to the genito-ventral scutum and, in addition to the anal pore, bears the three anal spines. On the genito-ventral and anal scutum there are scale-like markings and transverse lines. A pair of endopodal plates is borne on the soft integument above the level of the third pair of genito-ventral spines. On the soft integument of the venter there are five pairs of spines.

Leg ii stouter and shorter than the others. On the venter of coxa i there is a stout spine or spur as in the male; on coxa ii there is a tooth-like spur on the dorso-anterior edge; on coxa iii there is a stout spur on the posterior edge ventrally. The stigmal pore lies level with coxa iii and the peritreme extends anteriorly to the middle of coxa i.

The dorsal scutum is shown in dotted line and will be seen to cover the anterior third of the body leaving exposed a wide margin laterally and posteriorly. The dorsal spines are normal with the exception of the pair on the posterior end of the scutum which are longer and stouter than the others. The chelicera (Fig. 4*a*) has the fixed digit long, thin and curved, without teeth; the movable digit is provided with a curved distal tip and two teeth; at the base of the movable digit there is a fringe of small spines.

Host. A dermapteron (*Hemimerus talpoides* Walker, 1909).

Locality. Kabala, Sierra Leone, West Africa. Dr D. Eaton. 25 April 1950.

Measurements. ♂, 0·38 × 0·22 mm.; ♀, 0·47 × 0·30 mm.

Subfamily Trombiculinae Ewing, 1929

Genus **Acomatacarus** Ewing, 1942

Acomatacarus keegani n.sp.

The dorsal scutum (Fig. 5) shows its oblong shape, the anterior edge being slightly concave on each side of the median process; its lateral edge concave; the posterior edge is concave medially and is projected posteriorly behind each sensory seta. The antero-median setae are placed wide apart and below the level of the antero-lateral setae; the postero-lateral setae are wider apart than the antero-laterals. The sensory setae are long, slender and have lateral barbs on their distal third. The bases of the sensory setae are level with the postero-lateral setae and are about equi-distant from one another and from the lateral edge of the scutum. Flanking the scutum and posterior to a line drawn through these spines are the eye-plates, each bearing two eyes, the anterior eye being the larger.

The palpal claw (Fig. 6) is tridentate, the central or main prong being curved, stout and with a blunt tip, bearing an accessory claw on each side about half way down its length. Femoral seta with lateral barbs; genual seta with lateral barbs; lateral tibial seta long, slender and nude; dorsal tibial seta with lateral barbs. Galeal seta nude.

Chelicera (Fig. 7) with teeth on dorsal and ventral edges.

All tarsi with two claws and a long, simple empodium. Tarsus i (Fig. 8) with a short spur and at its base there is a short, inconspicuous microspur. The subterminala is long and slender. On the tibia there is a long, slender tibiala with the microtibiala close to its base. No mastitarsala on tarsus iii. On coxa i there are two spines ventrally; one spine on coxae ii and iii.

Dorsal setae about 114, arranged in transverse rows of 2, 14, 12, 8, 12, 16, 14, 12, 10, 8, 4 and 2. On the venter there are about one hundred small setae irregularly arranged.

Standard data. AW 90 μ, PW 105 μ, SB 30 μ, ASB 45 μ, PSB 28 μ, A–P 40 μ, AM 57 μ, AL 69 μ, PL 80 μ, Sens 70 μ, DS 60 μ, Humerals 90 μ, SD 74 μ.

Host. A cuckoo (*Centropus melanops*).

Locality. Samar, Tagaslian, Barongon. H. L. Keegan. 4 June 1948.

Genus **Trombicula** Berlese, 1904

Trombicula samara n.sp.

The dorsal scutum (Fig. 9) is oblong, wider than long, its anterior edge has a concavity on each side of the antero-median seta; the lateral edge is concave; the posterior edge is wider than the anterior and has two projections posterior to the bases of the sensory seta; the surface of the scutum is marked with a number of pores. The anterior median seta is shorter than the others and situated below the level of the antero-lateral setae; the antero-lateral setae are long and are placed in the angles of the scutum; the postero-lateral setae are longer than the others and occupy the postero-lateral angles of the scutum. The sensory setae are long, slender and barbed on their distal half. The bases of the sensory setae are placed wide apart and have a distinct ridge on their anterior edge. Flanking the scutum are the eye-plates, each bearing two eyes, of which the anterior eye is the larger, and is placed above the level of the postero-lateral setae.

The palpal claw (Fig. 10) is tridentate, the central prong being stout, curved, with two accessory claws. Femoral seta with lateral barbs; genual seta with lateral barbs; dorsal tibial seta nude; lateral tibial seta nude. Galeal seta nude. Chelicera with a dorsal tooth.

All tarsi with two claws and a long, simple empodium. On tarsus i (Fig. 11) the spur is long and tapering, about half the length of the tarsus. A small cone-like microspur is placed close to the base of the parasubterminala, which lies between it

and the subterminala. On the second segment of leg i the tibiala is short with a microtibiala and also a second sensory spur. On tarsus iii there are no mastitarsala. One seta on each coxa, ventrally.

Dorsal setae, about forty-six, arranged in transverse rows of 2, 8, 4, 8, 8, 4, 6, 4 and 2. On the venter there are some twenty-eight arranged in rows of 2, 2, 2, 2, 6, 6, 4, 2 and 2.

Standard data. AW 80 μ, PW 85 μ, SB 30 μ, ASB 32 μ, PSB 15 μ, A–P 48 μ, AM 40 μ, AL 48 μ, PL 60 μ, Sens 60 μ, DS 55 μ, Humerals 62 μ, SD 50 μ.

Host. A cuckoo (*Centropus melanops*).

Locality. Samar, Tagaslian, Barongon. H. L. Keegan. 4 June 1948.

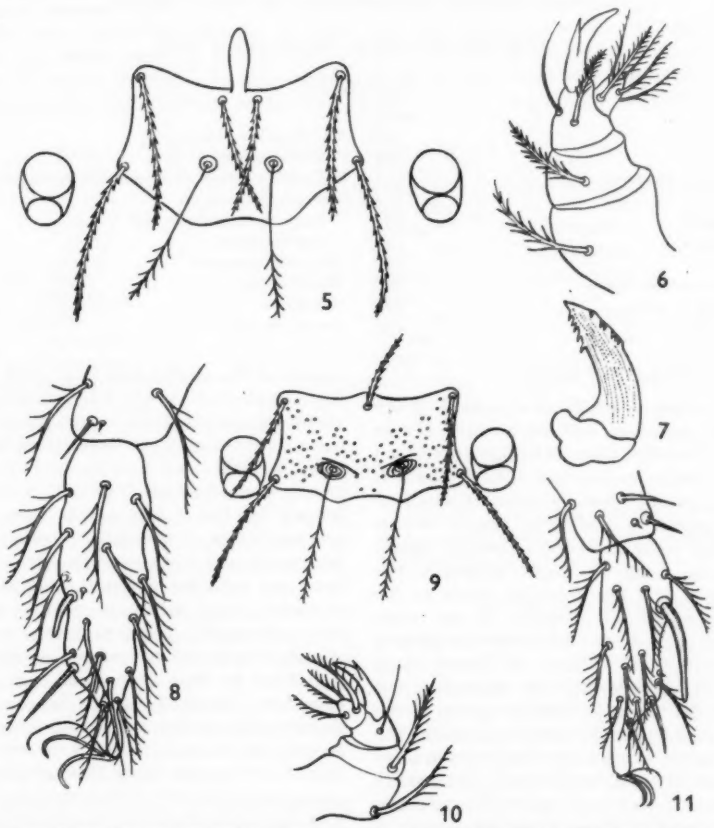


Fig. 5. *Acomatacarus keegani* n.sp. Dorsal scutum of larva.
Fig. 6. *A. keegani* n.sp. Palp of larva. Dorsal face.
Fig. 7. *A. keegani* n.sp. Chelicera of larva.
Fig. 8. *A. keegani* n.sp. Tarsus i of larva.
Fig. 9. *Trombicula samara* n.sp. Dorsal scutum of larva.
Fig. 10. *T. samara* n.sp. Palp of larva. Dorsal face.
Fig. 11. *T. samara* n.sp. Tarsus i of larva.

REFERENCES

EWING, H. E. (1942). Remarks on the taxonomy of some American chiggers (Trombiculinae), including the descriptions of new genera and species. *J. Parasit.* **28**, 485–93.

FONSECA, F. DA (1948). A monograph of the genera and species of Macronyssidae Oudemans, 1936 (synom. Liponissidae Vitzthum, 1931 (Acari)). *Proc. Zool. Soc. Lond.* **118**, 249–334.

HIRST, S. (1921). On some new parasitic mites. *Proc. Zool. Soc. Lond.* p. 769.

RADFORD, C. D. (1951). Five new species of mites (Acarina) parasitic on bats (Chiroptera). *Parasitology*, **41**, 97–101.

(*MS. received for publication* 20. II. 52.—Ed.)

ON THE LIFE HISTORY OF *ASCARIS DEVOSI* AND ITS DEVELOPMENT IN THE WHITE MOUSE AND THE DOMESTIC FERRET

By J. F. A. SPRENT, Ph.D., B.Sc., M.R.C.V.S. (*From the Department of Parasitology, Ontario Research Foundation, Toronto, Canada*)

(With Plate IV and 28 Figures in the Text)

CONTENTS

	PAGE		PAGE
Introduction	244	The 3rd stage larva	249
Methods	244	Development	249
Observations and results	244	Distribution of encapsulated larvae in mice	249
The egg	244	The 4th stage larva	251
The 1st stage larva	245	Experimental infection	251
The 2nd stage larva	245	Development	254
Development	245	The adult parasite	255
Distribution in mouse tissues	246	Discussion	256
Autopsy lesions in mice	248	Summary	257
Symptoms in mice	248	References	258

INTRODUCTION

Ascaris devosi occurs naturally as a parasite of the fisher (*Martes p. pennanti*) and the marten (*Martes a. americanus*). The adult parasite has been described in a previous publication (Sprent, 1952*b*), and it is proposed in this paper to describe some observations on the development of the larval stages of this parasite, as they were observed following experimental infection, using the white mouse as the intermediate host and the domestic ferret as the final host. Experimental infection of the white mouse with the eggs of *Ascaris devosi* has already been reported (Sprent, 1952*a*), *A. devosi* being referred to as *A. mustelarum*. By estimating the distribution of the larvae in various tissues of the mouse, it was found that the larvae penetrated the wall of the intestine, whence they migrated in large numbers to various parts of the body, particularly the subcutaneous tissues and general musculature. It was evident that in this regard the life history of *A. devosi* differed quite remarkably from that of *A. lumbricoides*. The purpose of the experiments reported in the present paper was to follow the development of the larval stages of *A. devosi* and to establish whether the somatic migration of the larvae in the mouse makes possible the utilization of this animal as a true intermediate host in the life history of the parasite.

METHODS

Culture of eggs, infection of mice and the collection of larvae from their tissues was carried out as previously described (Sprent, 1952*a*). The various species of Mustelidae used as possible final hosts for the parasite were infected by allowing them to kill and devour infected mice. Administration of gelatin capsules containing the larvae from digested mouse tissue was also used as a method of infection. The ferrets were obtained from a local breeder and as judged by faecal egg counts were found to be uniformly free of helminth infection upon arrival. At autopsy none of these animals was observed to have any parasitic infection other than as a result of experimental infection. Skunks were obtained from Algonquin Park, Ontario, an area where *A. columnaris* had never been collected, and were found to be free of ascaris eggs; *Physaloptera maxillaris* occurred in the stomach, *Filaroides mustelarum* in the lungs, and *Skrjabingylus chitwood orum* in the frontal sinuses. A marten was obtained from the Chapleau Game Preserve: repeated faecal examination showed trematode eggs, but no nematode eggs were observed. Larvae were preserved in a hot aqueous solution containing the following ingredients: 1% acetic acid, 5% glycerin, 6% formaldehyde (40%). Before staining they were treated for 15 min. with 2% potassium hydroxide or 0·5% sodium hypochlorite solution to soften the cuticle. They were stained in Delafield's haematoxylin and mounted, after dehydration, in beechwood creosote.

OBSERVATIONS AND RESULTS

The egg (Pl. IV, fig. 1)

The eggs which are ready to be passed out of the female lie in the undivided portion of the uterus,

only a few occurring in the vagina. They are oval in outline and measure 0·058–0·077 (average 0·064) mm. in length, and 0·051–0·061 (average 0·056) mm. in width. The outer of the three egg membranes is covered with minute pits and elevations; it is evidently sticky as the eggs usually become coated with particulate matter and the eggs themselves are readily adherent. The middle membrane is a transparent smooth shell, while the inner membrane has wavy striations parallel to the surface of the egg which make it appear darker than the other two layers. Unfertilized eggs appear to lack the differentiation into three egg membranes, only the middle one being evident. In old cultures the egg membranes appear to become thinner, particularly the outer layer, which seems to be worn away and partially replaced by adhering debris.

Cleavage begins as soon as the eggs are removed from the uterus and placed in a suitable environment such as the charcoal culture. For the first 6 days the ovum undergoes cleavage, at the end of which time a tadpole-shaped embryo is formed. By 8 days the 1st stage larva is developed and begins to move (Fig. 1).

The 1st stage larva (Fig. 1)

Between the 8th and the 12th day after culturing the eggs, the 1st stage larva becomes fully differentiated. This larva bears three well-defined lips; the oesophagus is relatively long, being about 31–35 % of the body length; the intestine, terminated by the rectum, consists of a row of seven rectangular cells each packed with spherical granules. The excretory pore is discernible in the older larvae on a minute elevation of the body surface ventral to the oesophagus, and the genital rudiment lies between the intestine and the body wall on the ventral side. In the youngest larvae the cells of the body wall, particularly in the intestinal region, are full of granules; these appear to be depleted as the larva grows. The intestinal cells retain their granules and become, in the older larvae, sharply differentiated. As the larva develops it becomes more slender, the width of the body at the end of the oesophagus diminishing from about 7 % to about 4 % of the body length. The cultures were examined at various times after 12 days and it was observed that on each occasion about 25–30 % of the larvae were enclosed in a cuticular sheath (Fig. 2), the remainder being free. The 1st moult thus occurs in the egg, many of the 2nd stage larvae evidently emerging from the old cuticle before the egg hatches. Eggs were found to be infective for mice at 12 days after culturing and were still infective a year later, at which time the average length of the larvae was 0·279 mm. The cultures were kept moist at a room temperature of 68–80° F. For measurements of the 1st stage larva, see Table 1.

Table 1. *Body measurements (mm.) of larvae of* A. devosi *from mice*

	1st stage (15)* 12 days after cleavage	2nd stage (98) 1–9 days after infection	3rd stage (26) 12 days to 6 months after infection
Length	0·200–0·308	0·215–1·150	1·125–1·912
Width (widest point)	0·012–0·016	0·014–0·042	0·045–0·065
Width (% of body length)	7–4	6–4	4–3·5
Oesophagus length	0·070–0·089	0·077–0·201	0·175–0·262
Oesophagus (% of body length)	35–31	33–16	16–12
Excretory pore to anterior end	0·042–0·054	0·045–0·105	0·101–0·126
Genital rudiment to posterior end	0·074–0·093	0·070–0·350	0·332–0·577
Anus to tip of tail	0·023–0·026	0·023–0·056	0·052–0·070
Cuticular corrugations (average width near posterior end of oesophagus)	—	—	0·0035

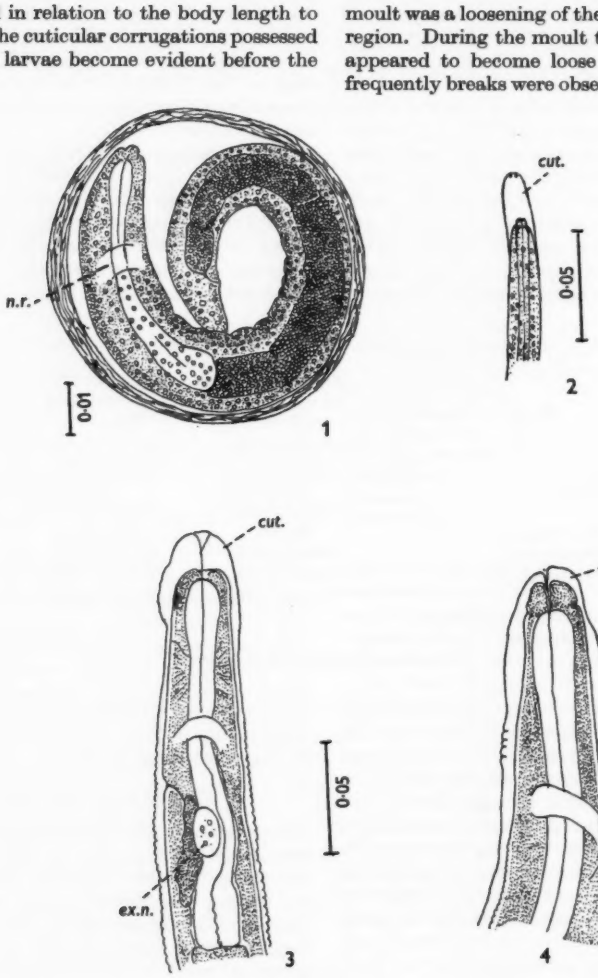
* Number of specimens examined.

The 2nd stage larva (Figs. 5–10)

Development. After the egg hatches in the alimentary canal of the mouse, the 2nd stage larva penetrates the intestinal wall. During its 2nd stage the larva grows considerably in length and width (Fig. 27). The original single row of intestinal cells becomes split longitudinally so that a narrow intestinal lumen is formed, bordered by two rows of elongate cells (Fig. 8). Later by cell division the intestine becomes multicellular, being lined by cuboidal cells (Fig. 10). The cells of the body wall, as well as the intestinal cells, become filled with spherical granules reminiscent of the early 1st stage larva. The lateral lines become prominent during this stage, they commence just in front of the nerve ring and reach to the posterior end; each consists of a cuticular ridge associated with a double row of subcuticular nuclei. Evidently it is during the 2nd stage that the food material is stored whereby the 3rd stage larva can survive a long period of inactivity when it is encapsulated in the tissues. During the 2nd stage the larva develops a conspicuous tooth-like projection at the anterior end; it disappears before the 2nd moult. The oesophagus

becomes reduced in relation to the body length to about 16 % and the cuticular corrugations possessed by the 3rd stage larvae become evident before the moult.

moult was a loosening of the cuticle behind the head region. During the moult the cells of the intestine appeared to become loose from one another and frequently breaks were observed, particularly at the



Figs. 1–4

Fig. 1. First stage larva in egg from 8-day-old culture (outer and middle layer of shell removed by sodium hypochloride solution).

Fig. 2. Second stage larva in sheath of 1st stage expressed from egg from 12-day-old culture.

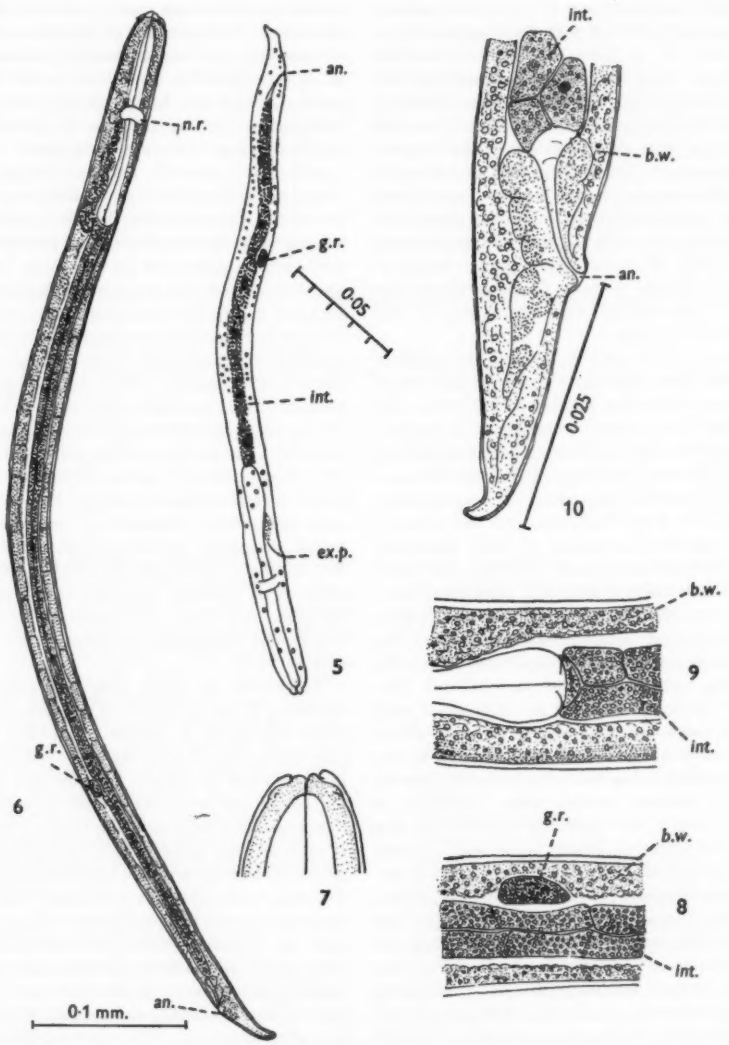
Fig. 3. Third stage larva in sheath of 2nd stage, from carcass of mouse 10 days after infection.

Fig. 4. Fourth stage larva in sheath of 3rd stage, from intestine of ferret 3 days after infection.

The time of occurrence of the 2nd moult (Fig. 3) was found to be somewhat variable. In one series of mice, moulting of larvae was observed as early as the 5th day after infection, the smallest moulting larvae being 0·56 mm. in length. In another series no moulting larvae were observed until the 8th day. In both series almost all of the larvae were moulting on the 10th day, but after 12 days few moulting larvae were observed. The first indication of the anterior end. The granules in the body wall and intestine became less numerous during the moult. No moulting forms were observed in the liver, lungs, brain, kidneys or mesenteric tissues, but only in the head, and in the anterior and posterior parts of the carcass. Table 1 gives measurements for the 2nd larval stage.

Distribution in mouse tissues. The distribution of the larvae in the different parts of the mouse's body,

at various times after infection, was investigated by killing two mice on each day after infection with 5000 eggs, and separating the body into the following parts: (i) intestinal wall, i.e. oesophagus, stomach, intestine and caecum, the contents of each being removed; (ii) liver; (iii) lungs; (iv) kidneys; (v) brain; (vi) heart; (vii) mesentery, spleen and associated structures. The rectum was discarded and the remainder of the mouse was designated 'the carcass' which included the head and the skeletal, muscular and connective tissues of the trunk and limbs; the feet were discarded. Fig. 28 shows the distribution



Figs. 5–10

Fig. 5. Second stage larva expressed from egg (12-month-old culture).

Fig. 6. Second stage larva from carcass of mouse 6 days after infection.

Fig. 7. Anterior end of 2nd stage larva.

Fig. 8. Genital rudiment of 2nd stage larva.

Fig. 9. Junction of intestine and oesophagus in 2nd stage larva.

Fig. 10. Tail of 2nd stage larva.

of the larvae in the intestinal wall, mesenteric tissues, liver, lungs and carcass for 14 days after infection. Larvae were collected also from the kidneys, heart and brain throughout the whole period, but in no case were there more than twenty, they are therefore not included in the chart. To investigate the distribution of the larvae in the various parts of the carcass itself during this period, a series of mice was infected with 2500 eggs and one killed each day for 8 days after infection. The carcasses of these mice were separated and divided into three parts by cutting off the head and dividing the trunk between the last thoracic and first lumbar vertebra. Throughout the duration of the experiment the percentage distribution of the larvae in these three parts was as follows: the head contained 3–10 % of the total number in the three parts, the back part contained 2–15 % and the front part contained 72–88 %. There was no regular change in distribution in relation to time. It was evident that the majority of the larvae were distributed to the anterior part of the body.

It will be observed from Fig. 28 that the number of larvae in the liver, mesenteric tissues and lungs rapidly decreases from the 1st day of infection, but that about 4 days after infection nearly all the larvae were distributed throughout the somatic musculature and connective tissues, few remaining in the thoracic and abdominal viscera. Regarding the course taken by these larvae in their migration to the somatic tissues, it is possible that some of them migrated directly out from the intestinal wall into the peritoneal cavity and thence into the retroperitoneal tissues. However, this cannot be the sole migratory pathway to the carcass because, as shown above, the part of the carcass in front of the diaphram invariably contained more larvae than the part behind. The first organs to be invaded after the intestinal wall were the liver and the mesenteric tissues, which suggests that most of the larvae were carried in the portal blood system, as has been shown to occur with the larvae of *Ascaris lumbricoides* (Ransom & Cram, 1921). Some are possibly carried in the lymphatics. The kidneys, brain, lungs and carcass were invaded within the first 3 days, and as it is unlikely that these tissues were reached through direct migration from the intestine, it is probable that the larvae had first travelled, by the blood, through the liver and the lungs. The disappearance of larvae from the liver is thus probably due to their being carried away in the blood stream. Disappearance from the mesenteric tissues may be partly by the blood and lymphatics, and perhaps to some extent by direct migration into the retroperitoneal tissues of the carcass. When the larvae of *A. devosi* arrived at the lungs during the first 3 days of infection, after which time the numbers were insignificant, their maximum diameter was about 0·021 mm. In contrast, most of the larvae of *A. lumbricoides* arrived in the lungs of mice between the 6th and 8th days after infection (Sprent, 1949, 1952*a*), by which time their width had reached 0·038–0·084 mm. According to Roberts (1934) they proceed to moult at about this time in the lungs. It seems very probable that the larvae of *A. devosi*, because they get to the lungs earlier and are consequently smaller, are able to pass through the pulmonary circulation into the arterial circulation (somatic migration), whereas the larvae of *A. lumbricoides* being larger at the time they pass through the lungs undergo a predominantly tracheal migration, because they break out of the capillaries into the alveolar spaces.

Autopsy lesions in mice. Autopsy lesions were observed in forty-four mice, killed at various times between 1 day and 6 months after infection. The first lesions to be observed were red patches, streaks and spots which appeared on the liver, lungs, intestine and kidneys on the 1st day after infection. On the 2nd day the lungs were uniformly dark red and the red patches had also appeared on the surface of the cerebral hemispheres, and on the subcutaneous tissues. Yamaguchi (1925) has shown with other ascaris infections that these red spots and patches on the various organs result from the larvae breaking out of the capillaries causing petechial haemorrhage. The haemorrhagic spots continued to appear on these various tissues until the 8th day, after which time they were replaced by grey streaks of scar tissue especially apparent on the kidneys and liver. As already mentioned, the 2nd stage larvae commenced to undergo their moult about 8 days after infection and their encapsulation in the diaphram, heart, and subcutaneous tissues was first observed at this time.

Symptoms in mice. The pulmonary symptoms resulting from infection of the mouse with the 2nd stage larvae of *A. devosi* resembled those seen in infections with the larvae of *A. lumbricoides* except in their time of onset. In infection with larvae of the latter parasite the mouse usually commenced dyspnoea and disinclination to move or feed between 6 and 8 days after infection, because the larvae arrive at this time in the lungs causing considerable damage to the alveolar walls and resulting in intra-alveolar haemorrhage (Sprent & Chen, 1949). In the case of infection with the larvae of *A. devosi*, symptoms commenced 2 days after infection and became more severe on the 3rd day; if the infection was heavy enough the mouse usually died on the 4th day after infection. If the mouse lived through the 5th day it usually survived, provided that nervous symptoms did not develop. Nervous symptoms resulting from infection with the larvae of *A. devosi* occurred only very rarely compared with similar infections of *A. columnaris*. They were observed in only two mice and occurred about 3 weeks after

infection. They consisted of locomotor inco-ordination and circum-ambulation, the mice constantly holding the head on one side. Such symptoms have been described by Tiner (1949, 1951) as occurring frequently in mice infected with the larvae of *A. columnaris* from the raccoon.

The time of onset of pulmonary congestion and the resulting symptoms of loss of appetite, dyspnoea and prostration, together with the above-mentioned findings on the distribution of larvae in the various tissues are evidence to show that for 4 days after infection the larvae of *A. devosi* pass from the liver to the lungs and thence into the arterial circulation. While it is not intended to consider in this publication the histological changes occurring in the lungs and other tissues during migration of the larvae, it must be pointed out that if, as suggested above, the larvae gain access to the arterial circulation by merely passing through the pulmonary capillaries from the pulmonary arteries to the pulmonary veins, it would not be expected that such severe damage would be done to the lungs. The behaviour of the larvae in the lungs during these 4 days and its effect on the lung tissues awaits further investigation, although it has been established (Sprent, 1952*a*) that the larvae do not reach the intestine in significant numbers and thus probably do not travel up the trachea. However, it is possible that they may break into the alveolae from the pulmonary capillaries and return into the venules. This behaviour was suggested by Fülleborn (1925) to explain the appearance of strongyloides larvae in the somatic tissues.

The 3rd stage larva (Figs. 11–17)

Development. Most of the 3rd stage larvae had emerged from the 2nd moult by 12 days after infection. The 3rd stage larva is more slender than previous stages and its anatomical details are more readily discerned. It differs from the previous stages in that the lips are larger, the lateral lines are more prominent, the nerve ring is more distinct, the nucleus of the left excretory lobe is very conspicuous and the intestinal wall possesses several rows of polygonal cells. These larvae soon become encapsulated and may remain more or less dormant for many months. As shown in Fig. 27, growth continues, although further morphological changes were not evident, between 3 weeks and 6 months after infection. The mature 3rd stage larva has further reduced the relative length of the oesophagus to about 12% of the body length and the cuticular corrugations are well developed. The genital rudiment shows no change. For measurements see Table 1.

Distribution of encapsulated larvae in mice. Table 2 shows the distribution of larvae in the tissues of five mice killed at 1, 2, 3, 4 and 6 months respectively after infection with eggs of *A. devosi*. The larvae collected from these mice were vigorously motile after they were liberated by peptic digestion. The carcass was divided into three parts as described previously and it is evident that the majority of the encapsulated larvae also occur in the front part of the carcass. Table 3 shows how the larvae of *A. devosi* differ from those of *A. columnaris* in their distribution. The larvae of *A. columnaris* appeared to remain to a greater extent in the thoracic and abdominal viscera than the larvae of *A. devosi*. This confirms a previous observation of this difference in migratory behaviour between the two species (Sprent 1952*a*).

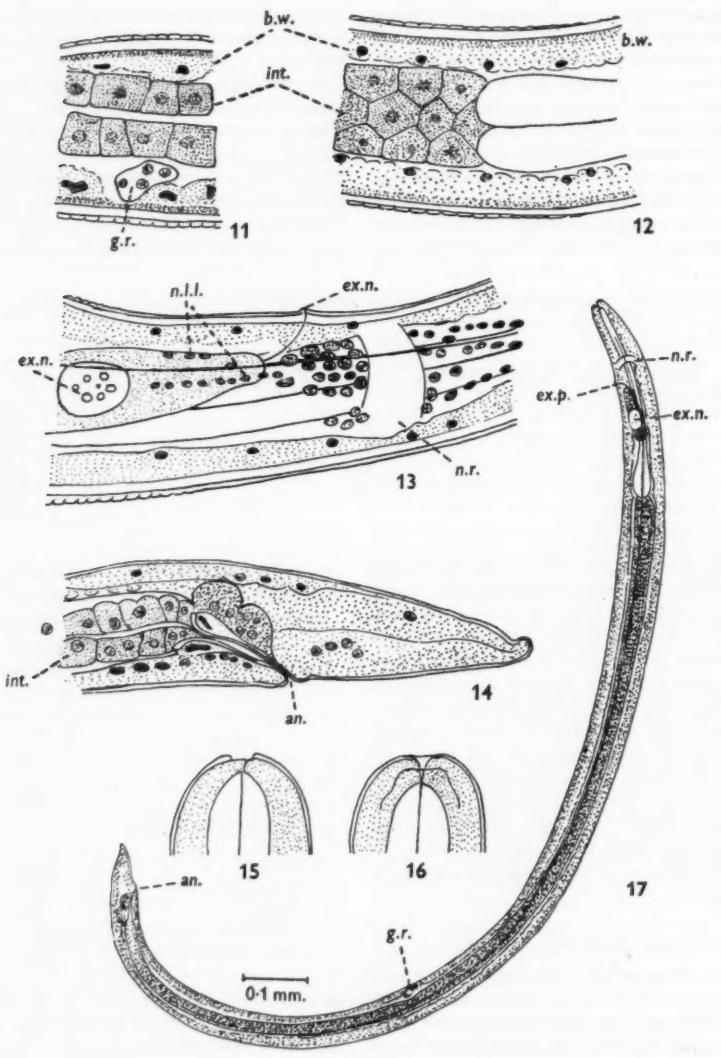
Table 2. *Distribution of encapsulated larvae in five mice killed* 1–6 *months after infection with* A. devosi

	Heart	Lungs	Liver	Mesentery and spleen	Brain	Kidney	Int. wall	Head	Carcass: Front	Carcass: Back
Ascaris devosi (5000)										
1 month	4	0	0	0	0	1	0	120	2240	340
2 months	0	8	0	0	0	0	0	254	2100	252
3 months	4	6	15	0	0	0	10	220	1400	160
4 months	0	0	0	0	0	0	0	140	1640	340
6 months	10	10	0	0	0	0	0	240	2500	160

Table 3. *Distribution of encapsulated larvae in ten mice infected* 1–6 *months previously with* 5000 *eggs of* A. devosi *and* A. columnaris

	Average percentage of total number of larvae in mouse	
	A. devosi	*A. columnaris*
Heart	0·14	1·74
Lungs	0·23	0·75
Liver	0·12	9·32
Mesentery and spleen	0	1·26
Brain	0	0·13
Kidney	0·01	0·26
Intestinal wall	0·08	1·40
Head	8·00	15·00
Front part of carcass	81·13	54·46
Back part of carcass	10·28	15·69

After 3 weeks the white nodules which imprison the 3rd stage larvae were very conspicuous. They were easily seen at autopsy, particularly in the muscles of the jaw, neck, shoulders and back (Pl. IV, fig. 2). They also occurred in the subcutaneous connective tissue of the thorax, abdomen and limbs, and in the diaphragm. The nodules consist of a layer of fibroblasts and connective tissue and a core of inflammatory cells surrounding the imprisoned larva (Pl. IV, fig. 3). The cellular changes occurring as a result of larval migration will be described in a separate publication.



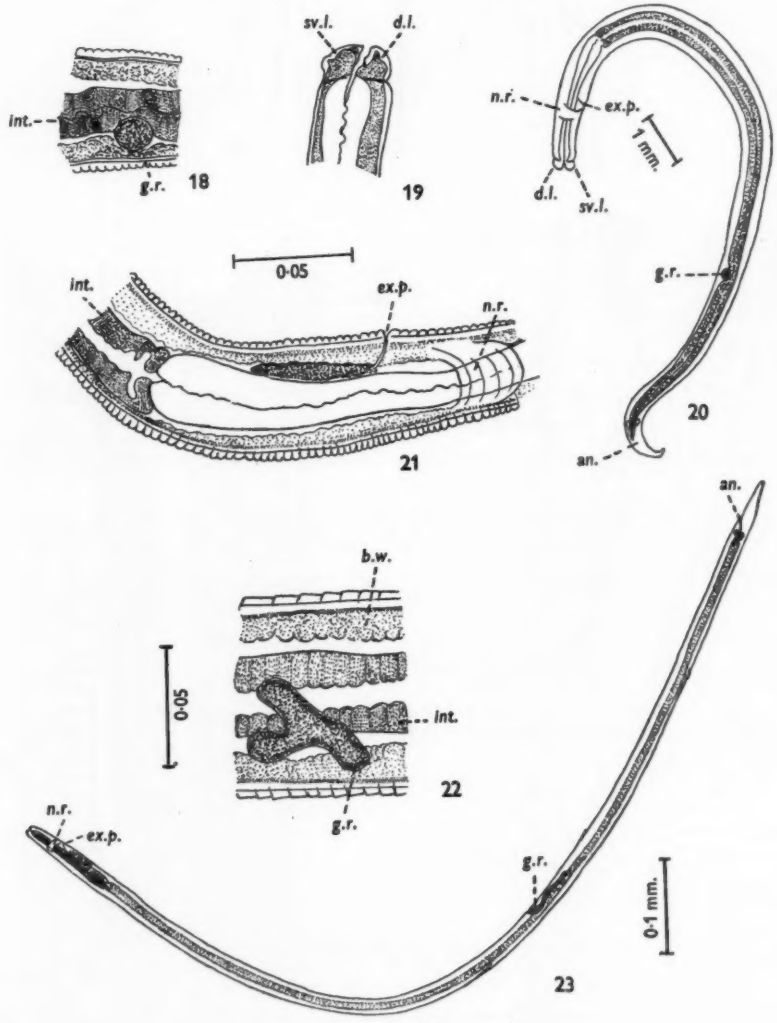
Figs. 11–17

Fig. 11. Genital rudiment of 3rd stage larva.
Fig. 12. Junction of oesophagus and intestine in 3rd stage larva.
Fig. 13. Mid-oesophageal region of 3rd stage larva.
Fig. 14. Tail of 3rd stage larva.
Fig. 15. Anterior end of 3rd stage larva (lateral).
Fig. 16. Anterior end of 3rd stage larva (dorsal).
Fig. 17. Third stage larva.

The 4th stage larva (Figs. 18–26)

Experimental infection. Thirteen ferrets, a skunk and a marten were used in the following experiments:

(1) Two adult ferrets (F 1–F 2) born during the previous year, were each given 10,000 eggs of *A. devosi* by oral administration. F 1 was killed after 8 days and F 2 after a month. The stomach and intestinal contents, as well as various other tissues after digestion, were searched for larvae. Three larvae were recovered from F 1, the kidneys and intestinal wall containing one 2nd stage larva each;



Figs. 18–23

Fig. 18. Genital rudiment of 4th stage larva from ferret, 3 days after infection.

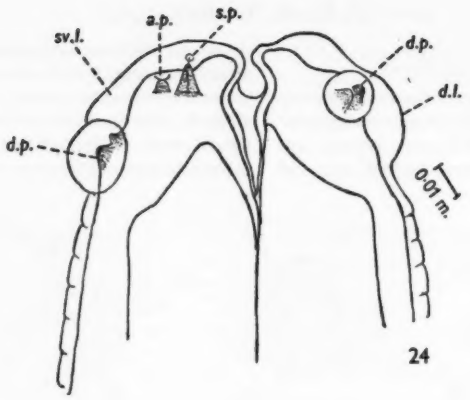
Fig. 19. Lips of 4th stage larva from ferret, 3 days after infection.

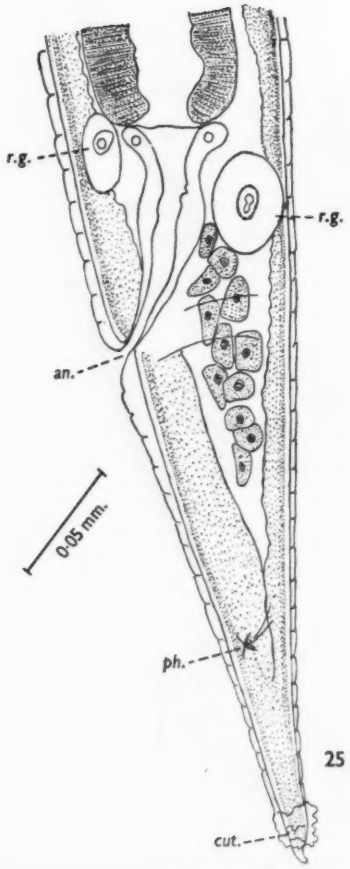
Fig. 20. Fourth stage larva from ferret, 3 days after infection.

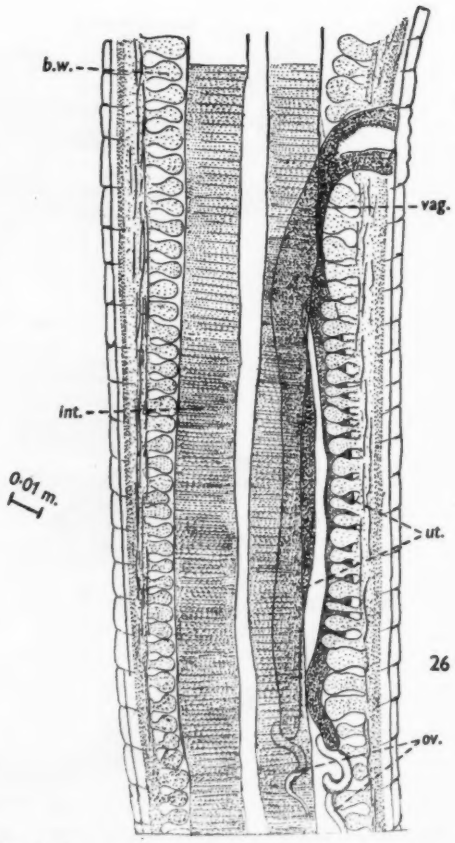
Fig. 21. Oesophagus of 4th stage larva, from intestine of ferret 3 days after infection.

Fig. 22. Genital rudiment of 4th stage larva from ferret 7 days after infection.

Fig. 23. Fourth stage larva from ferret 16 days after infection.







Figs. 24–6

Fig. 24. Anterior end of 4th stage larva from ferret 21 days after infection (lateral view).

Fig. 25. Tail of female 4th stage larva from ferret 21 days after infection.

Fig. 26. Development of female reproductive organs in 21-day-old female 4th stage larva from ferret.

the liver and lungs contained no larvae. F2 harboured two larvae in the intestinal wall, two larvae in the heart wall, and five larvae in the carcass; they were all 3rd stage larvae; the liver, of *A. devosi*. Its faeces were collected over the next 4 days and about 500 vigorously motile larvae were recovered. The larvae recovered over the first 2 days were all 3rd stage larvae but within the next 2 days

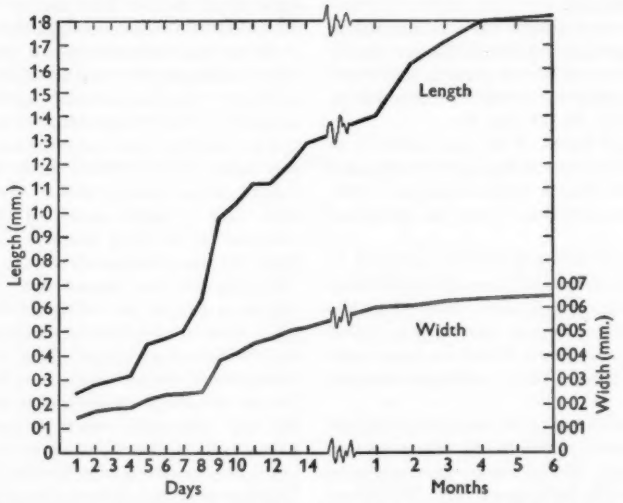


Fig. 27. Chart showing growth of larvae of *A. devosi* in experimentally infected white mice.

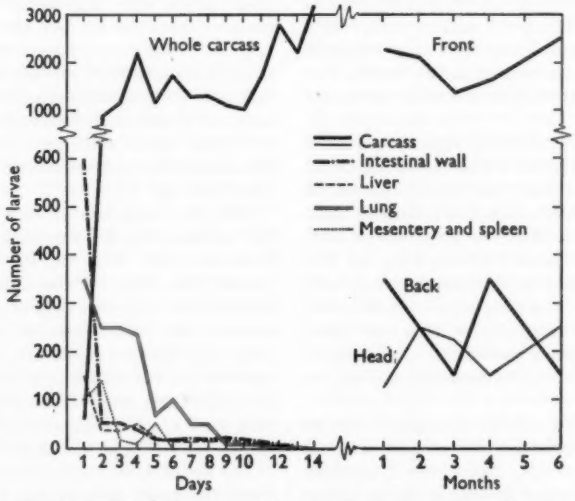


Fig. 28. Chart showing distribution of larvae of *A. devosi* among various tissues of experimentally infected white mice.

lungs, kidneys and intestinal contents contained no larvae.

(2) One adult ferret (F4) was allowed to kill and eat a mouse infected 2 months previously with eggs some moulting larvae and a few 4th stage larvae were collected.

(3) Four adult ferrets (F8–F11) were each given 500 larvae after the latter had been obtained by

digestion from infected mice. The larvae were administered in gelatin capsules. Two were killed after 2 weeks and two after 3 weeks. No larvae were recovered from the stomach or intestinal contents or from the digested intestinal wall. The faeces were not examined.

(4) One ferret (F 12) 6 weeks old was allowed to kill and eat a mouse infected 3 months previously with eggs of *A. devosi*. After 16 days it was killed and hundreds of 4th stage larva were recovered from the intestinal contents (Pl. IV, fig. 4).

(5) One 6-week-old ferret (F 13) was allowed to kill and eat a mouse infected 25 days previously with eggs of *A. devosi*. It died 3 weeks later and three 4th stage larvae were recovered from the intestinal contents.

(6) One 6-week-old ferret (F 14) was allowed to kill and eat a mouse infected 3 months previously with eggs of *A. devosi*. Eggs were observed in the faeces 41 days after infection and it was killed 8 weeks after infection. Seven female and two male *A. devosi* were recovered. Their measurements are shown in Table 5.

(7) One 6-week-old ferret (F 15) was allowed to kill and eat three infected mice. Each infection was separated by a month. The larvae in the three mice were 8, 10 and 12 weeks old respectively. No larvae were recovered from the ferret when it was killed 7 days after the last infection.

(8) One 6-month-old ferret (F 16) was allowed to kill and eat a mouse infected 4 months previously with the eggs of *A. devosi*. Three days after infection one 4th stage larvae was passed in the faeces. The ferret was killed 28 days after infection; no larvae were recovered.

(9) One 6-month-old ferret (F 17) was infected in the same way as F 16, but killed 1 week after infection. One 4th stage larva was recovered from the intestinal contents and two from the digested intestinal wall.

(10) One adult ferret (F 5) was given about fifty 4th stage larvae from ferret F 12 (see Exp. 4). The larvae were administered by the mouth in a gelatin capsule. Five months later this ferret was observed to be passing eggs of *A. devosi* and when it was killed, two male and eight female parasites were collected from the intestine, their measurements are shown in Table 5.

(11) One adult ferret (F 3) was given larvae digested from the tissues of an infected mouse whose carcass had been kept at $-20°$ C. for 2 months. Many living 3rd stage larvae and some moulting forms were collected from the faeces 1–2 days later. The ferret was not killed.

(12) One young skunk (4–8 weeks) was allowed to kill and eat a mouse infected 2 months previously with eggs of *A. devosi*. No ascaris eggs were observed in the faeces until 8 weeks later when it was killed and three male and two female specimens of *A. devosi* were recovered from the intestine. These specimens were mentioned in a previous publication (Sprent, 1952*b*).

(13) One adult marten was allowed to kill and eat three mice all infected 3 months previously with eggs of *A. devosi*. The faeces were examined for 12 weeks but no ascaris eggs were observed.

From these experiments it was evident that an adult marten and several adult ferrets, i.e. those born in the previous year, were not infected by oral administration of embryonated eggs or larvae digested from the carcasses of mice, or after eating infected mouse carcasses. In contrast, three out of four young ferrets, being about 6 weeks old at the time of infection, and a single skunk of the same age were infected by allowing them to kill and eat infected mice. It was established that in adult ferrets many 3rd stage larvae passed out in the faeces alive within a day or two of infection, and it appeared likely that the adult ferret's intestine was unsuitable for the 3rd stage larvae. Exp. 10 showed that the adult ferret's intestine was not, however, unsuitable for the 4th stage larvae and it seems probable that an age immunity exists in infection with this parasite, being particularly active on the 3rd stage when they are freed from encapsulation by digestion. Further work would be necessary to establish this.

Development. It was evident from the specimens obtained in the above experiments that the 3rd stage larvae were digested from the carcass of the mouse within the 1st day after its ingestion. Many of these larvae were passed out in the faeces, some of them alive and free, others still encapsulated. The larvae which maintained a hold in the intestine grew to about 2 mm. and then proceeded to moult about 3–4 days after infection (Fig. 4). The smallest 4th stage larva was recovered 3 days after infection (see Table 4).

The 4th stage larvae were distinguishable from the 3rd stage by the constriction separating the lips from the body (Fig. 19), by the conspicuous rectal glands (Fig. 25), by the appearance of a definite lumen in the anterior part of the intestine (Fig. 20), and by the more distinct cuticular corrugations (Fig. 21). The youngest 4th stage larvae appeared shorter and rather stumpy compared with the fully developed 3rd stage larvae (Fig. 20), but at the end of a week considerable elongation had taken place, the larvae being at this time 3–4 mm. in length.

The lips of the 4th stage larva were well demarcated from the body and during this stage the double papillae appeared, two on the dorsal lip and one on each subventral lip. In larvae 21 days after infection the subventral lips each showed in addition a single papilla and an amphidial pore on the lateral aspect (Fig. 24). The cervical papillae were also observed at this time situated laterally just behind the nerve ring. In female larvae the phasmids were apparent

at 16 days after infection on each side of the tail (Fig. 25). The oesophagus resembled that of the adult, there being no distinct neck to the bulb (Fig. 21). In the youngest 4th stage larvae the intestinal cells were cuboidal, but as the larvae grew, these cells became columnar (Fig. 22). The rectum appeared to be flattened dorso-ventrally; there are three rectal glands each with a conspicuous nucleus, two glands lie subventral to the rectum and a larger one lies dorsally (Fig. 25).

The adult parasite (Pl. IV, fig. 5)

Mature specimens were obtained from ferrets at 2 months and at 6 months after infection; their measurements are given in Table 5. The adult female parasite differed from the 4th stage larva in the presence of the dentigerous ridges on the lips, in the absence of lateral alae along the body, in the relatively narrower corrugations of the cuticle, and in the presence of an open vulva.

Table 4. *Body measurements (mm.) of 4th stage larvae of* A. devosi *from ferrets*

Time after infection	3 days	7 days	16 days	21 days
Number of specimens	1	3	3	3
Length	1·375	3·5–3·8	9·4–12·7	11·23–16·25
Width	0·056	0·095–0·010	0·154–0·210	0·184–0·238
Width (% of length)	4	3	2	—
Width of head	0·029	0·038	0·068–0·087	0·070–0·091
Oesophagus—length	0·245	0·385–0·429	1·00–1·19	0·986–1·31
Oesophagus (% of body length)	18	12–11	10–9	10–9
Oesophagus (width of bulb)	0·031	0·040–0·049	0·084–0·099	0·085–0·131
Excretory pore to anterior end	0·122	0·166–0·184	0·283–0·338	0·315–0·346
Genital rudiment to posterior end	0·385	1·31–1·35	—	—
Vulva* to anterior end	—	—	4·92–6·50	5·76–8·08
Vulva (% of body length)	—	—	55–51	51–50
Anus to tip of tail	0·044	0·078–0·105	0·192–0·236	0·208–0·231
Cuticular corrugations at posterior end of oesophagus	0·002	0·005–0·007	0·012–0·017	0·019

* The vulva is closed during the 4th stage.

The distance between the anterior end of the body and the genital rudiment, in the youngest 4th stage larva observed, was about 70 % of the total body length (Fig. 20). In three specimens collected at 7 days this distance was reduced to about 60 % of the body length and three outgrowths had developed from the genital rudiment (Fig. 22). At 16 days it was evident in larvae over 7 mm long that these outgrowths had developed into the vagina and the two uteri (Fig. 26). As the larvae grew, the relative distance between the vagina and the anterior end decreased. In the oldest 4th stage larva observed (3 weeks), this distance was 50 % of the body length. It was evident that sexual differentiation becomes apparent in the female larvae in the 1st week after infection of the ferret and consists of a forward movement of the genital rudiment and its development into the vagina and uterus. The early development of the male larva was not followed. The larvae obtained at 16 days after infection varied considerably in size (5–12 mm.) (Pl. IV, fig. 4). In those measuring over 7 mm. the vagina was already formed. In the smallest specimens (3–7 mm.) no genital rudiment or reproductive organs were observed, possibly they were males. The three specimens obtained at 21 days were all females.

It was observed that the position of the vulva relative to the total body length moves in an anterior direction as growth continues. As mentioned above, the distance between the vulva and the anterior end in the longest 4th stage larva was 50 %. In the 2-month-old females from ferret F 14 the ratio was 34–37 %. In two mature females taken from an experimentally infected skunk 54 days after infection the ratio varied between 34 and 37 %. In the 6-month-old specimens from ferret 5, the ratio was 29–33 %. It was evident that in no case did this ratio approach that found for *A. columnaris*, i.e. 19–28 % (Sprent, 1952*b*).

Considerable contraction occurred when living worms were fixed for preservation. Accordingly the measurements of the body length, width and the position of the vulva in Table 5 applies to living worms. For technical reasons the other measurements apply to fixed and cleared worms. When comparing these measurements with those of *A. devosi* from the natural hosts (Sprent, 1952*b*) it should be borne in mind that the latter specimens were dead at the time of preservation and little or no shrinkage was observed. The measurements given in Table 5 show that, while *A. devosi* was already sexually mature at 2 months after infection.

Table 5. *Body measurements (mm.) of adult specimens of* A. devosi *from ferrets*

	2 months		6 months	
	Male	Female	Male	Female
Specimens examined	2	7	1	6
Length*	70–75	125–140	110–120	188–238
Width*	—	—	1·56	1·96–2·55
Width of head (ventral view)	0·296–0·315	0·322–0·385	0·439	0·477–0·531
Dorsal lip (width)	0·203–0·217	0·245–0·266	0·277	0·346–0·377
Dorsal lip (length)	0·105–0·140	0·126–0·175	0·223	0·246–0·316
Denticles (width of base)	0·0033–0·0034	0·0042–0·0043	0·0045	0·0045–0·0065
Oesophagus length	3·64–3·69	4·00–4·27	4·90	5·580–6·075
Oesophagus length (% body length)	5	3	4	3
Cervical papillae to anterior end	0·826–0·875	0·861–0·966	1·150	1·371–1·524
Excretory pore to anterior end	0·630–0·644	0·665–0·735	1·04	1·016–1·140
Vulva to anterior end*	—	41·00–47·00	—	62·00–74·00
Vulva to anterior end (% body length)	—	34–37	—	29–33
Anus to tail	—	0·546–0·665	—	0·539–0·847
Phasmids to tail	—	0·098–0·196	—	0·146–0·231
Cloaca to tail	0·346	—	0·439	—
Spicules	0·346–0·385	—	0·462	—
Cuticular corrugations (width)†	—	—	0·009	0·009–0·019

* Body length, width and the relative position of the vulva was estimated on living specimens, other measurements after fixation.

† Measured near the posterior end of the oesophagus.

growth continued after this time. The length of the 6-month-old specimens approached that of the longest specimens recovered from the fisher or marten and were longer than any specimen of *A. columnaris* recovered by the writer from the skunk. The two female specimens obtained from the experimentally infected skunk (Exp. 12) measured 152 mm., the three males measured 80–95 mm.

All the adult specimens of *A. devosi* collected from experimentally infected animals manifested quite distinctly the characters by which this species has been distinguished and separated from *A. columnaris*, i.e. the shape of the denticles in the dentigerous ridges, and the position of the vulva and the length of the spicules in relation to the body length (Sprent, 1952*b*).

DISCUSSION

It was suggested previously (Sprent, 1952*a*) that the ascaris parasites of terrestrial mammals fall into two categories according to the migratory behaviour of their larvae in mice. In the first category were placed *A. lumbricoides* of man and pig, and *Parascaris equorum*, because their larval migrations resulted in their appearance in the intestinal contents following a tracheal migration from the lungs. In the second category were placed *Ascaris columnaris*, *A. devosi* and *Toxocara canis*, most of whose larvae migrated from the lungs to the somatic tissues, few or none appearing in the intestinal contents. A third category should be added for *Toxascaris leonina* and *T. transfuga*, whose larvae most frequently became encapsulated in the wall of the intestine. It was further suggested that these variations in the migratory behaviour may be of significance in determining the mode of infection with these parasites. A criticism which may be levelled at this suggestion is that such differentiation is based upon observations made in an abnormal host, i.e. the mouse, the inference being that when the larvae occur in their natural hosts their migratory behaviour may be uniform. The elucidation of the life history of *Ascaris devosi*, together with the discovery by Tiner (1949) that skunks could be infected with *A. columnaris* by feeding them mice harbouring the encapsulated larvae of this parasite, strongly suggests that for these two parasites the rodent is not an abnormal host but is a true intermediate host. Moreover, failure to obtain any appreciable migration following the ingestion of infective eggs of *A. devosi* by the ferret and of *A. columnaris* by the skunk (unpublished) is evidence that an intermediate host may be obligatory with these two parasites.

The somatic migration of the larvae in the body of the mouse is evidently as fundamental and necessary a phenomenon in the life history of *A. devosi* as is the tracheal migration in the life history of *A. lumbricoides*. A point of considerable theoretical interest is whether the somatic migration or the tracheal migration is the more primitive. In other words, whether *A. devosi* is an older parasite in the evolutionary sense than *A. lumbricoides*.

Fülleborn (1920) at first considered that the

larvae of *A. lumbricoides* underwent their pulmonary migrations because they were descended from skin-penetrating ancestors. Later (1927) he concluded that its life history has been derived through the loss of an intermediate host. He considered that the original mode of infection may have involved an intermediate host in the same way as has been shown for ascaris parasites of fish, birds and marine mammals. He suggested that the present mode of infection resulted from the definitive host acting as an intermediate host, as well as the final host; the larvae, instead of remaining in the tissues, making their way from the lungs to the trachea and thence to the intestine. The intermediate host was thus dispensed with in accordance with the feeding habit of the definitive host.

Vinnitzky (1945) disagreed with the later hypothesis of Fülleborn and believed that the ascaris group owe their origin to a form whose larvae gained access by skin penetration. From such an early mode of infection he considered that three types of life history were developed. (i) The *A. lumbricoides* type, in which the egg has developed a resistant eggshell adapted for oral infection, although the larvae still undergo a lung migration. (ii) The *Ascaridia lineata* type where the infection is through ingestion of eggs but there is no longer any lung migration. (iii) The *Porrocaecum decipiens* type where the eggs are ingested by an intermediate host, in which the larvae become encapsulated, and the definitive host is infected by eating the intermediate host harbouring encapsulated larvae.

The discovery of ascaris parasites of terrestrial mammals, whose life history involves an intermediate host, sheds further light on this controversy, particularly when the larval migrations are considered. The essential differences between the migratory behaviour of the larvae of *Ascaris devosi* and *A. lumbricoides* is that the larvae of the former arrive at the lungs very soon after they have penetrated the intestinal wall and before they have undergone the 2nd moult. At this time the width of the larvae is possibly small enough to allow them to pass through into the arterial circulation. The larvae of *A. lumbricoides* on the other hand do not reach the lung capillaries until most of them are probably too large to pass into the arterial circulation and thus gain access to the alveolae.

It seems likely that the tracheal migration of certain ascaris species, e.g. *A. lumbricoides* and *Parascaris equorum* may be regarded as an adaptation towards loss of the carnivorous habit, on the part of the host, by a parasite which originally developed in carnivorous mammals and utilized their prey as intermediate hosts. Furthermore it is likely that the occurrence of these parasites in carnivorous terrestrial mammals, such as the Mustelidae, preceded their spread to such hosts as man, pig, and horse.

SUMMARY

The development of *Ascaris devosi*, a parasite of the fisher and marten, was followed from the egg to the adult stage using the white mouse and the ferret as the intermediate and final hosts respectively. The eggs contained motile 1st stage larvae 6 days after cleavage and were infective at 12 days, the 1st moult having already occurred. The eggs remained infective for at least 1 year. The 2nd stage larva after hatching from the egg in the intestine of the mouse passes through the intestinal wall to the liver and mesenteric tissues. At 3 days after infection they were recovered from the heart, lungs, brain, kidneys and from the carcass. The larvae grow and store food material during the 2nd stage and between 8 and 12 days after infection they undergo the 2nd moult. The mouse shows the most severe pulmonary symptoms on the 3rd and 4th days after infection, the lungs showing complete red hepatization at this time. The 3rd stage larva is relatively inactive and becomes encapsulated in various tissues, particularly in the muscular and subcutaneous tissues of the neck, shoulders and thorax. The chief developmental changes, apart from growth, which occur in the 2nd and 3rd stage larvae are: (i) the intestine develops from a single row of cells to a multi-cellular tube; (ii) the body cavity appears; (iii) the excretory lobes appear, the nucleus on the left side becoming prominent at the end of the 2nd stage; (iv) the cuticle shows transverse striations at the end of the 2nd stage; (v) the lateral lines become prominent.

The encapsulated 3rd stage larvae remained alive for at least six months in the tissues of mice and at 25 days after infection of the mouse they were able to develop in the young ferret following killing and ingestion of the mouse. No infection of ferrets was obtained through oral administration of embryonated eggs or 3rd stage larvae digested from mouse tissues.

The 3rd moult occurred in the intestine of the young ferret 3–4 days after infection; in adult ferrets the 3rd stage larvae were evidently unable to gain a hold and were passed out in the faeces. In the next 2–3 weeks the larva grew from about 2 to 16 mm. the 4th moult occurring between 2 and 3 weeks after infection. During the 4th stage the lips develop into the adult form and sexual differentiation occurs. In the female the genital rudiment moves forward and becomes differentiated into the vagina, uteri and ovaries. The vulva remains closed throughout the 4th stage.

The adult parasites had developed to sexual maturity by 56 days after infection, but they con-

tinued to grow and were considerably longer at 6 months after infection. The position of the vulva relative to the body length was found to move from about midway along the body in the 4th stage larva to a position at the junction of the anterior and middle third of the body in the mature adult.

The life history of this parasite is discussed in relation to that of *A. lumbricoides* and other species. It is considered that the life history of *A. devosi*, requiring as it does a true intermediate host for its completion, provides further information on the evolutionary development of the ascaris group. This work accordingly supports the hypothesis that the earliest members of this group utilized an intermediate host and does not support that which supposes that ascaris parasites are descended from skin-penetrating forms.

During this investigation the writer has benefited considerably from correspondence with Dr J. D. Tiner, Department of Zoology, University of Illinois, Urbana, Illinois, U.S.A. His thanks are also due to Dr H. B. Speakman and Dr A. M. Fallis for their encouragement, guidance and help.

This work was supported by the Province of Ontario on the recommendation of the Research Council of Ontario.

Grateful acknowledgement is made to Mr Cliff Smith of the Connaught Medical Research Laboratories of the University of Toronto for photographic work.

REFERENCES

FÜLLEBORN, F. (1920). *Arch. Schiffs- u. Tropenhyg.* **24**, 340–47.

FÜLLEBORN, F. (1925). *Arch. Schiffs- u. Tropenhyg.* **29**, Beihefte (3), 5–100.

FÜLLEBORN, F. (1927). *Arch Schiffs- u. Tropenhyg.* **31**, Beihefte (2), 151–202.

RANSOM, B. H. & CRAM, E. B. (1921). *Amer. J. Trop. Med.* **1**, 129–59.

ROBERTS, F. H. S. (1934). *Animal Health Sta. Bull. No.* 1, Yeerongpilly, Queensland.

SPRENT, J. F. A. & CHEN, H. H. (1949). *J. Infect. Dis.* **84**, 111–24.

SPRENT, J. F. A. (1952*a*). *J. Infect. Dis.* **90**, 165–176.

SPRENT, J. F. A. (1952*b*). *Proc. Helminth. Soc. Wash.* **19**, 27–37.

TINER, J. D. (1949). *J. Parasit.* **35**, suppl. p. 13.

TINER, J. D. (1951). *J. Parasit.* **37**, suppl. p. 21.

VINNITZKY, I. M. (1945). *C.R. (Doklady) de l'acad. des sci. des l'URSS.* **46**, 258–60.

YAMAGUCHI, S. (1925). *Arch. Schiffs- u. Tropenhyg.* **29**, 589–604.

EXPLANATION OF PLATE IV

Fig. 1. Egg before commencement of cleavage (× 350).

Fig. 2. Dorsal view of skinned mouse 2 months after infection showing encapsulated larvae.

Fig. 3. Section of 3rd stage larva encapsulated in the heart of a mouse (× 77).

Fig. 4. Fourth stage larvae from ferret's intestine 16 days after infection (natural size).

Fig. 5. Two male and one female adult *A. devosi* (natural size) from intestine of ferret 6 months after infection.

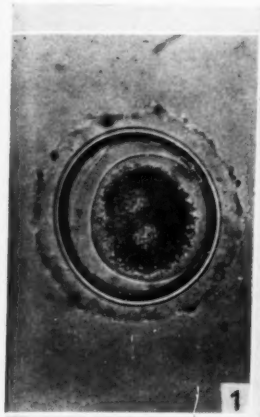
Key to lettering on Text-figures

Note. Magnification of Figs. 7–16 as in Fig. 10.

Figs. 11–14 were drawn from stained specimens, all other figures were from unstained specimens.

an. = anus; *a.p.* = amphidial pore; *b.w.* = cells of body wall; *cut.* = cuticular sheath; *d.l.* = dorsal lip; *d.p.* = double cephalic papilla; *ex.n.* = nucleus of excretory lobe; *ex.p.* = excretory pore; *g.r.* = genital rudiment; *int.* = cells of intestine; *n.r.* = nerve ring; *n.l.l.* = nuclei of lateral line; *ov.* = ovary; *ph.* = phasmid; *r.g.* = rectal gland; *sv.l.* = subventral lip; *s.p.* = single cephalic papilla; *ut.* = uterus; *vag.* = vagina.

(*MS. received for publication* 16. II. 1952.—Ed.)



PROBLEMS OF NOMENCLATURE FOR THE GROWTH OF ORGANISMS OF ONE SPECIES WITH AND WITHOUT ASSOCIATED ORGANISMS OF OTHER SPECIES

By ELLSWORTH C. DOUGHERTY, Ph.D., M.D.,* *Department of Zoology, University of California, Berkeley*†

A recent editorial in *Nature* (Anon., 1951) on current problems in parasitology has emphasized the importance of the cultivation of parasitic organisms free from their hosts and from other species. The increased interest of workers in this type of problem, and their expanded efforts with helminths (as reviewed by Read, 1950, for helminths in general, and by Hobson, 1948, for the nematodes), which have so far almost completely resisted *in vitro* cultivation through whole life cycles,‡ make highly desirable an assessment of the nomenclature to be used in describing various relationships between organisms, both in nature and in the laboratory.

Anyone who wishes to describe work on the cultivation of an organism either in the absence of living organisms of another species, or in specific combinations with one or more other known species, encounters the problem of nomenclature. This has been ably reviewed by Reyniers, Trexler, Ervin, Wagner, Luckey & Gordon (1949), who criticize the application of terms such as 'germ-free', 'pure', and 'sterile' to cultures of the first type as being inadequate or misleading, and basically limited to specific situations by reason of their negative application—that is, by reason of the fact that they describe a lack rather than a positive attribute and are hence not applicable to a general system of nomenclature. Reyniers *et al.* recognize the need for a systematic nomenclature based on a positive principle and propose a series of terms derived from a word coined by them—*gnotobiotics* (from γνωστός or γνωτός—'known'; and βίος—'life'); this may be defined as 'the science of growing organisms of one species under conditions where their environment contains either no, or only known, other species'.

The nomenclature of Reyniers *et al.* is comprehensive and fills a great need. In my opinion, however, it suffers from the fact that its terms are long and cumbersome and for that reason are unlikely of general acceptance. For example, growth of an organism in the absence of any other species is *monognotobiotic*, a rather unesthetic term. It is in marked contrast to the short, simple term *axenic* of Baker & Ferguson (1942) with the same meaning. Reyniers *et al.* have criticized this term as being based on a negative principle. Their objection makes sense as far as it goes, but I believe that it is possible to create a better system of nomenclature based upon the root ξένος (foreign, strange) of *axenic* that accomplishes the same end as that based on *gnotobiotics* and has the advantage of much greater euphony. This need not mean the abandonment of all the terms of the Reyniers group. I feel that *gnotobiotics*, is, for example, a good word and deserves application to the study of the growth of organisms taking place under certain precisely known conditions; it perhaps suffers by being an apparent analogue of 'antibiotics', a plural term for substances rather than a singular term for a science, but whether this will lead to sufficient confusion to prevent the acceptance of the former term can only be decided by time and usage.

First of all, I believe it important to define carefully the word *axenic*; this may be as follows: 'pertaining to growth of organisms of a single species in the absence of living organisms or living cells of any other species (also, strictly, in the absence of living cells of the organism itself except those in the intact living organism and as its gametes)'.

Using the terms *gnotobiotic* and *agnotobiotic* derived from the nomenclature of Reyniers *et al.*, but otherwise basing terminology upon the same root as *axenic*, one may derive the system of nomenclature presented in Table 1. Such an expression as 'two-membered culture' becomes 'monoxenic culture', and such a one as 'culture in the presence of an unknown mixed microbial flora' becomes 'agnotobiotic culture'. *Agnotobiotic* may not be a simple or euphonious term, but, as one word, it does the service of a long phrase and greatly simplifies the precise discussion of *axenic* or *synxenic* (that is, *gnotobiotic*) vs. *agnotobiotic* cultures. It should be noted that, whereas there is nothing in the etymology of *synxenic* to indicate that the 'foreign [life]' is

* Research Fellow of the American Cancer Society as recommended by the Committee on Growth of the National Research Council, 1949–52.

† The present communication was written while the author was a guest investigator at the Laboratoire de Zoologie, Faculté des Sciences, Université de Lyon, Lyon, France.

‡ Possible exceptions to this are semi-parasites of the nematode genus *Neoaplectana* Steiner, 1929, all occurring in insects (see Bovien, 1937; Glaser, McCoy & Girth, 1942).

known, I am here specifically defining the term in question and its dependent words—*monoxenic*, etc.—in this sense.

From *axenic**, as Reyniers *et al.* (1949) have already shown, one may derive the words *axenity* (state of being axenic), *axenize* (to render axenic), and *axenite* (an organism grown axenically). Similarly, from *synxenic* one can derive the homologous new terms *synxenity*, *synxenize* and *synxenite* (the principal organism being studied—the subsidiary organisms might be termed *xenites*). Conditions of axenity and synxenity can be collectively designated by the term *gnotobiosis*† of Reyniers' group.

Trager, 1948), and to a lesser degree vertebrates (see the *LOBUND Reports*, **1**, 1946; **2**, 1949). Workers such as Kidder & Dewey and the LOBUND group have clearly recognized and enunciated the concept that the study of the nutrition of an animal organism in the presence of other organisms cannot answer many fundamental questions; this in general has not been adequately recognized for the vertebrates, with which the vast preponderance of nutritional research done has been on the agnotobiotic assemblage of host and its symbiotic microflora.

Among animal symbiotes themselves much axenic work has been done with trypanosomes and their

Table. 1. *Terminology for growth or cultivation of an organism with and without associated organisms of other species.*

Terms	Number of associated organisms	Source of terms	Equivalent term in nomenclature of Reyniers *et al.* (1949)
Gnotobiotic	None, or known species only	Reyniers *et al.* (1949)	Same
Axenic	None	Baker & Ferguson (1942)	Monognotobiotic
Synxenic	One or more	New	None
Monoxenic‡	One	New	Dignotobiotic
Dixenic	Two	New	Trignotobiotic
Trixenic	Three	New	Tetragnotobiotic
Polyxenic	Several	New	Polygnotobiotic
Agnotobiotic	One or more species, at least one unknown (usually a mixed microbial flora)	Reyniers *et al.* (1949)	Same

‡ *Monoxenic* is not to be confused with *monoxenous*, a term describing a parasitic (or, more broadly, a symbiotic) organism with only one host in its life cycle (see Chitwood, 1941).

It is obvious that the system of nomenclature here suggested is applicable to parasitic and non-parasitic, or, as I should prefer to say, symbiotic and non-symbiotic, organisms alike. If one reviews the literature on gnotobiotics of animal organisms, one can scarcely help being impressed by the relatively primitive state of our knowledge in this field as opposed to extensive information on bacteria, molds, plant-like flagellates, and even the higher green plants. There are a few exceptional studies of an advanced nature on animals, dealing with the axenic growth and nutrition largely of certain free-living organisms—the ciliate genus *Tetrahymena* (see Kidder & Dewey, 1951), the fruit fly *Drosophila melanogaster* (see Hinton, Ellis & Noyes, 1951), the mosquito larva (see Golberg & De Meillon, 1948*a*, *b*;

* Pronounced *ā-zen'ic*.

† Actually, as defined by Reyniers *et al.*, *gnotobiosis* is synonymous with *axenity* only, even though *gnotobiotics* is proposed for the science of *both* axenic and synxenic cultivation; this seems inconsistent, and I feel that the term *gnotobiosis* should be used in the broader sense suggested here. Similarly, *gnotobiotize* has been proposed by Reyniers *et al.* with a definition identical with that of *axenize*; if this term is to be used at all, it should, in my estimation, refer collectively to the processes both of axenizing and of synxenizing.

relatives and with trichomonads (see Lwoff, 1951*a*). Despite long success in the *in vitro* agnotobiotic, and more recently monoxenic, culture of *Entamoeba histolytica*, this important species has steadfastly resisted efforts toward its indefinite axenic cultivation (Lwoff, 1951*b*). And so far no helminth symbiote of vertebrates has been reared *in vitro* through a life cycle including the symbiotic stages, even under agnotobiotic conditions.

The application, in the foregoing two paragraphs, of terminology proposed or supported here should, I believe, illustrate the utility of a system of nomenclature for the cultivation of organisms as isolated species and in various known combinations. In closing, I wish also to make reference to another nomenclatorial system—that for various intimate relations between organisms where a dependence of one on another is involved. Perhaps the loose usage of *parasitic* is too firmly entrenched in the literature to expect its strict limitation to conditions whereunder the host suffers while the associated organism benefits. However, it can perhaps be expected that the etymologically faulty and historically incorrect, but rather widespread, application of *symbiotic* to a mutually beneficial relationship of two or more organisms, properly

termed *mutualism*, can in time be eradicated. *Symbiosis*, as shown by Kirby (1941), who has discussed the problem at length, was orginally used as a broad, generic term; and I feel that it is desirable to apply it in this sense—that is, to be defined as, 'an intimate relationship between two (or rarely more) organisms such that one, the *symbiote*, gains nutrition or protection, or both, from another, the *host*'. Needless to say, the key here is 'intimate', and it is obvious that intergrading conditions between symbiosis and independent existence must exist. Nevertheless, the concept is manifestly a useful one, and symbiosis can be broadly divided into the three well-recognized categories: *commensalism* (where the host is for all practical purposes unaffected by the presence of the symbiote), *parasitism* (where the host is injured), and *mutualism* (where the host is benefited).

REFERENCES

ANONYMOUS (1951). [Editorial.] Future of parasitology. *Nature, Lond.*, **168**, 527–9.

BAKER, J. A. & FERGUSON, M. S. (1942). Growth of platyfish (*Platypoecilus maculatus*) free from bacteria and other micro-organisms. *Proc. Soc. Exp. Biol., N.Y.*, **51**, 116–19.

BOVIEN, P. (1937). Some types of association between nematodes and insects. *Vidensk. Medd. naturh. Foren., Kbh.*, **94**, 13–32.

CHITWOOD, B. G. (1941). Life history. General discussions. Ch. IV in: Christie, J. R. (ed.), *An Introduction to Nematology*, sect. II, pt. I., pp. 243–5. [Undated; date verified from other sources.]

GLASER, R. W., MCCOY, E. E. & GIRTH, H. B. (1942). The biology and culture of *Neoaplectana chresima*, a new nematode parasitic in insects. *J. Parasit.* **28**, 123–6.

GOLBERG, L. & DE MEILLON, B. (1948*a*). The nutrition of *Aedes aegypti* Linnaeus. 3. Lipid requirements. *Biochem. J.* **43**, 372–9.

GOLBERG, L. & DE MEILLON, B. (1948*b*). The nutrition of *Aedes aegypti* Linnaeus. 4. Protein and amino-acid requirements. *Biochem. J.* **43**, 379–87.

HINTON, T., ELLIS, J. & NOYES, D. T. (1951). An adenine requirement in a strain of *Drosophila*. *Proc. Nat. Acad. Sci., Wash.*, **37**, 293–9.

HOBSON, A. D. (1948). The physiology and cultivation in artificial media of nematodes parasitic in the alimentary tract of animals. *Parasitology*, **37**, 183–227.

KIDDER, G. W. & DEWEY, V. C. (1951). The biochemistry of ciliates in pure culture. In Lwoff, A. (ed.), *Biochemistry and Physiology of Protozoa*, **1**, 323–400. New York: Academic Press Inc.

KIRBY, H. (1941). Relationship between certain protozoa and other animals. In: Calkins, G. N. & Summers, F. M. (eds.), *Protozoa in Biological Research*, pp. 890–1008. New York: Columbia University Press.

LWOFF, M. (1951*a*). The nutrition of parasitic flagellates (Trypanosomidae, Trichomonadinae). In: Lwoff, A. ed.), *Biochemistry and Physiology of Protozoa*, **1**, 129–76. New York: Academic Press Inc.

LWOFF, M. (1951*b*). Nutrition of parasitic amebae. In: Lwoff, A. (ed.); *Biochemistry and Physiology of Protozoa*, **1**, 235–50. New York: Academic Press Inc.

READ, C. P., JR. (1950). The vertebrate small intestine as an environment for parasitic helminths. *Rice Inst. Pamphl.* **37**, 1–94.

REYNIERS, J. A., TREXLER, P. C., ERVIN, R. F., WAGNER. M., LUCKEY, T. D. & GORDON, H. A. (1949). The need for a uniform terminology in germ-free life studies. *LOBUND Rep.* **2**, 151–62.

TRAGER, W. (1948). Biotin and fat-soluble materials with biotin activity in the nutrition of mosquito larvae. *J. Biol. Chem.* **179**, 1211–33.

(*MS. received for publication* 4. III. 1952.—Ed.)

OBSERVATIONS UPON THE EFFECTS OF MEPACRINE AND OTHER SUBSTANCES ON *GIARDIA INTESTINALIS*

By MAIR E. M. THOMAS, B.Sc., M.B., Ch.B., D.P.H.
Public Health Laboratory, Edmonton

It is well known that mepacrine will eradicate a Giardial infection of the bowel (Brumpt, L., 1937, Galli-Valerio, 1937), and that while a 6-day course of treatment, using a daily dosage by mouth of from 0·1 to 0·3 g., according to age, is satisfactory, smaller doses or shorter courses of treatment are unreliable.

This account describes some observations made on the effect upon the parasite of exposure to different concentrations of the drug for various lengths of time and of its exposure under similar conditions to various other substances. Most of these studies were made *in vitro*; but some *in vivo* findings are described first, as the uncharacteristic appearance of the parasite seen occasionally in the fresh faeces seems likely to be related to the distortions produced experimentally.

IN VIVO OBSERVATIONS

Eighty-two cases of Giardial infection were investigated in this series. Of these, fifty-nine were drawn from children attending Day Nurseries in which outbreaks of diarrhoea had been attributed to *Giardia*, while the remaining twenty-three came from sporadic cases of diarrhoea investigated for general practitioners. In none of these cases were any other pathogens found. Faeces were examined on the day they were passed, usually within 4 hr. of defaecation.

Table 1. *Composition of series of cases*

Outbreaks	No. of infections	Acute diarrhoea	Diarrhoea during previous half-year
Nursery A	20 and 8 contacts	5	12
Nursery B	26 and 5 contacts	11	0
Sporadic cases			
Adults	10	7	1
Children, 1–5 years	13	8	2
Older children	No cases		

In only six of these eighty-two cases were vegetative forms observed; in the remainder cysts only were seen, even in fresh fluid stools from acute diarrhoeas. Five of the six cases in which vegetative forms were seen were adults with diarrhoea; the sixth was a 4-year-old child with diarrhoea.

All cases, diarrhoeal and symptomless, were prescribed a 6-day course of mepacrine. A 3-day interval after the end of treatment was advised, and then 'clearance' specimens of faeces were examined until at least three negatives had been obtained.

RESULTS OF TREATMENT

In seventy-three cases no parasites were found after treatment had been completed; but in nine cases (five nursery and four sporadic) abnormal round or oval bodies with refractile walls were seen in the faeces. These were of the size of *Giardia* cysts (8–15 μ), but not of the same morphology. The content of each 'cyst' was shrunken, so that only a half to two-thirds of the internal space was occupied. The material appeared granular or structureless and yellow. It stained very darkly with eosin and with Lugol's iodine. Bodies of this kind have not been seen by us in faeces from untreated cases of *Giardia* infection, whether acute or chronic.

These bodies were seen up to the 6th day after treatment, but not subsequently, in the faeces of six patients whose infection did not relapse; but in another case they were followed and, in a further two cases, accompanied by typical *Giardia* cysts.

Enquiry revealed that these three relapsed cases had taken only a portion of their prescribed dosage of mepacrine. They cleared up completely when a second course of treatment was given. One of these cases was observed daily during a second course of treatment. Shrunken forms were not seen before treatment was commenced, but were seen in increasing numbers relative to normal cysts from the 2nd to 4th days inclusive. On the 5th day shrunken forms, but no normal cysts, were seen, thereafter no

parasites of any kind were seen on repeated examinations.

It seemed probable that the refractile-walled bodies which had been seen were degenerate *Giardia* cysts; so experiments were performed to observe the effect of mepacrine *in vitro*.

A few parallel experiments with other chemicals were then made to ascertain whether the observed effects of mepacrine were similar to those produced by other agents.

The action of some substances commonly used in the cleaning of infected premises and utensils was also tested.

IN VITRO OBSERVATIONS

Technique

In making this series of observations a single simple technique was employed throughout. Samples of faeces from six patients heavily infested with *Giardia* cysts were taken, and approximately 0·5 g. quantities were mixed with 1 ml. volumes of a number of test substances contained in small screw-cap bottles. The contents of these bottles were examined microscopically at intervals. Some of the tests were kept in duplicate at two temperatures, 20 and 37° C.

Two microscopic preparations from each test were examined on each occasion, one in saline, and one in eosin. Wet iodine staining was also used except with the mepacrine preparations.

Eosin was used on the assumption that only non-viable *Giardia* cells would take up the stain. Hoare (1949) found the method a practical test of the viability of cysts of amoebae and other protozoa. It is not known whether all non-staining *Giardia* cysts are in fact capable of survival, since there is no reliable method of culturing the parasite. Observations made during this study suggest that some substances may interfere with eosin staining and that preliminary yellow staining by mepacrine may mask or delay the process.

From each microscopic preparation ten consecutive cysts were drawn to scale on paper. A note was made of the average number of cysts per high power field so that reductions in numbers could be recorded as well as changes in appearance. The 'test substances' used were as follows:

(1) Mepacrine suspended in 0·85 % NaCl at the following range of percentage w/v concentrations: 0·062, 0·125, 0·25, 0·5, 1·0, 2·0 % at a final pH neutral to litmus.

(2) Mepacrine as in (1) above, but at final pH 6·0 and at pH 8·0.

(3) Isotonic (0·85 %) NaCl in aqueous solution.

(4) Hypertonic (6 %) NaCl in aqueous solution.

Aqueous solutions of the following substances:

(5) Distilled water.

(6) Hydrochloric acid, 0·013, 0·052, 0·13, 0·26, 0·52 %.

(7) Sodium hydroxide, 0·015 %, 0·062, 0·25, 1·0, 2·0, 4·0 %.

(8) Acetic acid 10 and 5 %.

(9) 50 % of a commercial detergent.

(10) Lysol 0·2, 1·0 and 5 %.

(11) Dettol 0·2, 1·0 and 5 %.

An additional experiment was made in which vegetative forms of the parasite were exposed to concentrations of mepacrine, of 0·6–2·5 % in isotonic saline, and examined in parallel in isotonic saline alone.

EXPERIMENTAL RESULTS

Arrangement of tables

Details of the observations made are presented in Tables 2–6. In each table the result of observations on cysts from the first sample of faeces studied is given, followed in brackets by the counts obtained when the experiment was repeated using faeces from other patients. In no experiment was there any considerable difference between the results obtained with cysts from different patients. The number given refers to those cysts which showed the relevant abnormality out of ten consecutive cysts studied microscopically. Where reduction in the total number of cysts took place, the number of cysts destroyed was calculated from the average number of cysts per microscopic field seen on the first examination of the fresh preparation.

The results of exposure of cysts to some intermediate concentrations of test substances, and also some counts made at intermediate times, are omitted for the sake of brevity.

Table 2. *Types of degeneration recorded*

	Nature of degeneration	Preparations in which it was sooner or later observed
1	Increase in proportion of cysts whose protoplasm stained with eosin	All preparations
2	Staining of nuclei but not cytoplasm, with eosin	NaOH, acetic acid
3	Staining of protoplasm with mepacrine	Mepacrine
4	A. Pronounced shrinkage of protoplasm within intact cyst wall	Detergent, mepacrine, 6 % NaCl
	B. Slight shrinkage of protoplasm within intact cyst wall	0·85 % NaCl, HCl, Distilled H_2O
5	Distortion of cyst outline	Lysol
6	Damage to and dissolving of cyst wall	NaOH
7	Destruction of cysts	NaOH, distilled water

Types of degeneration in cysts

Apart from the actual destruction of cysts, several types of degeneration were seen during this study. Some of these changes developed sooner than others, and some developed only, or much more rapidly, in the presence of relatively high concentrations of the test substance. Often two or more types of degeneration occurred together. Where fewer than 100% of cysts were affected at the first inspection, the proportion showing abnormalities was found to increase steadily with the passage of time.

Effects of mepacrine on cysts

When 0·5 g. of a sample of faeces containing cysts of *Giardia intestinalis* was suspended in 1 ml. of physiological saline and incubated at 37° C., no apparent alteration in the structure of the cysts took place within 24 hr., but after 4 days approximately half the cysts present lost their typical structure, and after 6 days no normal cysts remained and there was some reduction in the number of cysts. The cyst wall was unaltered, but the internal structures lost definition, became subject to eosin staining, and sometimes (perhaps as a later stage of the same changes) shrank from the cyst wall to form a granular mass surrounded by a clear space.

When the same specimen was kept under similar conditions, but with the addition of mepacrine, a similar but more rapid and pronounced shrinkage took place, together with yellow staining of the protoplasm. Eosin staining was hard to assess owing to the masking effect of this yellow dye; but some cysts clearly did take up eosin.

The proportion of affected cysts increased with the passage of time, and the rate of degeneration rose with the concentration of mepacrine present. Over a period of 4 days there was no reduction in the total number of cysts, but after 6 days there was some reduction. These mepacrine effects were not altered by varying the pH of the preparations between pH6 and pH8.

In this table it is seen that cysts exposed to concentrations of mepacrine of 0·25% and over shrank more rapidly than did those suspended in isotonic saline, and that the proportion of cysts affected at any given time increased with the concentration of mepacrine present.

Hypertonic saline had a pronounced effect on up to 40% of cysts after 4 hr., but the remaining 60% did not degenerate any more rapidly than those in isotonic saline. This was unlike the steady progressive degeneration seen in the presence of mepacrine.

The yellow staining of mepacrine obscured the effect of eosin. Probably mepacrine staining is itself lethal. After 4 days' incubation from 90 to 100% cysts exposed to mepacrine (0·06% strength and over) were yellow-stained, whereas only 40–80% of cysts in saline took up eosin. It is probably not misleading to compare these figures as indices of non-viability.

Effect of mepacrine on vegetative forms

When vegetative forms of the parasite were exposed to mepacrine in strengths of 0·6, 1·25 and 2% in isotonic saline, motility was lost within 5 min. Preparations in isotonic saline alone were still motile after 5 min. but had lost their motility after 30 min. Later (after 24 hr.) morphological changes developed in both saline and mepacrine preparations, and in the latter gross distortions and shrinkages were seen.

Effect of mepacrine upon cysts compared with that of other substances

Observations after 4 hr., shown in Table 5, are compared, in Table 6, which is similarly arranged, with those after 48 hr. incubation of the preparations. These tables are given in full below because no two substances tested had exactly the same effect.

Each substance used in these experiments produced a characteristic set of changes in the appearance of cysts, and in most cases the proportion

Table 3. *Number of cysts affected by protoplasmic shrinkage after incubation in isotonic saline* (0·85 NaCl), *in mepacrine suspensions, and in hypertonic saline* (6% NaCl)

Giardia cysts: shrinkage of protoplasm in mepacrine and in saline preparation. No. of cysts affected out of ten observed consecutively. (The figures in brackets refer to observations made on other samples of infected faeces.)

Time	Temp. (° C.)	0·85% NaCl isotonic	Mepacrine 0·06	Strength in g./100 ml. 0·25	2·0	6% NaCl hypertonic
½ hr.	20	0 (0, 0, 1)	0 (0, 0, 0)	0 (0, 0, 1)	0 (0, 0, 0)	—
4 hr.	37	1 (0)	0 (0)	1 (0)	1 (0)	4 (3)
24 hr.	37	0 (0, 0, 0)	0 (0, 0, 0)	1 (10, 2)	3 (2, 1, 4)	—
48 hr.	37	4 (2)	3 (3)	6 (4)	10 (8)	4 (2)
4 days	37	6 (4, 4, 6)	6 (6, 5, 7)	9 (8, 9, 8)	9 (10, 10, 9)	6 (4)
6 days	37	10 (10, 10, 10)	10 (10, 10, 10)	10 (10, 10, 10)	10 (10, 10, 10)	10 (10)

Table 4. *Result of attempts to stain cysts with eosin after 24 hr. and after 4 days' incubation*

Giardia cysts: staining by eosin and mepacrine. (The figures in brackets refer to observations made on other samples of infected faeces.)

			Strength of mepacrine suspension as g./100 ml.					
			0·06	0·25	2·0	0·06	0·25	2·0
Time of incubation	Temp. (° C.)	Red 0·85 % NaCl	Yellow-orange			Red-orange		
24 hr.	37	2 (0, 1, 1)	0 (0, 0, 0)	1 (1, 0, 1)	3 (2, 2, 4)	0 (0, 0, 0)	0 (0, 0, 0)	0 (0, 0, 0)
4 days	37	8 (6, 4, 6)	9 (9, 9, 10)	10 (9, 9, 10)	10 (10, 10, 10)	0 (0, 0, 0)	3 (2, 13)	4 (3, 4, 4)

Table 5. Giardia *cysts: changes seen in cysts after 4 hr. incubation at 37° C. in preparations of different test substances*

(Number affected out of ten consecutive cysts observed. The figures in brackets refer to observations made on other samples of infected faeces.)

Concentration of test substance in gm./100 ml.		Reduction in cysts visible ('cysts destroyed')	Non-viable cysts (eosin stained)	Shrinkage of protoplasm within cyst	Other effects
Mepacrine	0·06	0 (0)	0? (0)	0 (0)	—
	0·25	0 (0)	0? (0)	1 (0)	—
	2·0	0 (0)	0? (0)	1 (0)	—
NaCl	0·85	0 (0)	1 (0)	1 (0)	—
	6·0	0 (0)	10 (6)	4 (3)	—
Distilled water		3 (1)	4 (4)	1 (0)	—
HCl	0·013	0 (0)	0 (0)	0 (0)	
	0·052	0 (0)	2 (2)	0 (0)	'Survival' of 40–80 % in HCl of gastric juice strength
	0·13	0 (0)	3 (3)	0 (0)	
	0·26	0 (0)	7 (5)	0 (0)	
	0·52	0 (0)	10 (8)	0 (0)	
NaOH	0·015	0 (0)	6 (4)	0 (0)	Nuclei stained with eosin. Only 30 % had visible walls to cysts
	0·062	4 (4)	6 (6)	0 (0)	40 % destroyed, only half of the remainder had visible walls to cysts
	1·0	6 (6)	2 (–)	0 (0)	60 % destroyed, remainder empty 'ghost cysts'
	2·0	10 (10)	– (–)	– (–)	All cysts destroyed

affected rose steadily as the time of incubation or the concentration of test substance was increased.

When cysts were kept in isotonic saline at 37° C. there was a gradual increase in the number susceptible to staining by eosin, followed by a moderate degree of shrinkage within the cyst wall. All cysts were so affected after 6 days and some had disintegrated.

The effect of *mepacrine* has been described. There was a slowly developing yellow staining of protoplasm (which obscured eosin staining) followed by a shrinkage of protoplasm within the cyst wall, which developed more rapidly than the 'spontaneous' shrinkage in isotonic saline and proceeded to affect nearly 100 % of cysts after 48 hr. Very few cysts disintegrated even after 6 days.

The majority of cysts suspended in hypertonic saline stained with eosin after 4 hr., and 30–40 % showed shrinkage of protoplasm at that stage; but this was not so rapidly progressive as in the presence of mepacrine.

No protoplasmic shrinkage was produced by distilled water, but there was an increase in the proportion of cysts staining with eosin, and a 20 % reduction in the number present after 4 hr.

Cysts suspended in dilute hydrochloric acid stained with eosin after 48 hr., but it was noted that from 40 to 80 % of cysts survived 4 hr. exposure to concentrations within the limits of human gastric acidity. After 48 hr. a few cysts were affected by slight protoplasmic shrinkage.

Most of the cysts suspended in dilute acetic acid stained with eosin after 48 hr. In many the nuclei stained quite darkly and this sometimes contrasted with unstained protoplasm.

Even dilute solutions of sodium hydroxide had

Table 6. Giardia *cysts: changes seen in cysts after* 48 *hr. incubation at* 37° *C. in preparations of test substances*

(Number affected out of ten consecutive cysts observed. The figures in brackets refer to observations made on another sample of infected faeces.)

Concentration of test substance in gm./100 ml.		Reduction in cysts visible ('cysts destroyed')	Non-viable cysts (eosin stained)	Shrinkage of protoplasm within cyst	Other effects
Mepacrine	0·06	0 (0)	?	3 (3)	70 % orange
	0·25	0 (0)	?	6 (4)	90 % orange
	2·0	0 (0)	2+? (2)	10 (8)	80 % orange, 20 % red
NaCl	0·85	0 (0)	4 (4)	4 (2)	—
	6·0	0 (0)	10 (8)	4 (2)	—
Distilled water		2 (2)	6 (6)	1 cyst (0)	—
HCl	0·013	0 (0)	8 (6)	1 (0)	Degree of protoplasmic shrinkage only slight
	0·052	0 (0)	9 (9)	1 (0)	
	0·13	0 (0)	10 (8)	1 (1)	
	0·26	0 (0)	10 (10)	4 (2)	
	0·52	0 (0)	10 (10)	3 (3)	
NaOH	0·015	9 (9)			The few remaining cysts were faint and incomplete, many without recognizable protoplasm
	0·062	10 (8)			
	1·0	10 (10)			
	2·0	10 (10)			
Acetic acid	1·0	0 (0)	10 (8)	2 (2)	Nuclei stain darkly with eosin. Degree of protoplasmic shrinkage slight
	5·0	0 (0)	10 (6)	4 (2)	
Detergent	50·0	0 (0)	0 (0) None stained	8 (7)	Very pronounced shrinkage of protoplasm

Table 7. Giardia *cysts: changes in cysts after exposure to Lysol and to Dettol*

(Number affected out of ten consecutive cysts observed.)

Concentration of test substance in gm./100 ml. aqueous solution		Non-viable cysts (eosin stained) ½ hr. at 20° C.	Non-viable cysts (eosin stained) 48 hr. at 37° C.	Distortion of wall 48 hr. at 37° C.
Lysol	0·2	6 (4)	10 (8)	0 (0)
	1·0	10 (10)	10 (10)	1 (1)
	5·0	10 (10)	10 (10)	7 (5)
Dettol	0·2	6 (0)	8 (6)	0 (0)
	1·0	10 (10)	10 (10)	0 (0)
	5·0	10 (10)	10 (10)	0 (0)

a damaging effect, and most cysts stained with eosin after 4 hr. In some the nuclei stained darker than the protoplasm. The walls of the cysts were rapidly eroded. After that the protoplasm became transparent and a 'ghost cell' was produced.

A detergent tested at 50 % strength and observed after 48 hr. incubation, caused a very pronounced shrinkage of protoplasm within 70–80 % of cysts, but none of the cysts took up eosin. This was probably due not to continuing viability but to interference with staining. There was no destruction of cysts.

Table 7 shows the effect of Lysol and of Dettol. There was a rapid lethal action (eosin staining) but no destruction of cysts and no shrinkage of protoplasm.

DISCUSSION

(*a*) *Susceptibility of cysts of* Giardia *to degeneration and damage*

The vegetative forms of *Giardia intestinalis* die soon after they are passed in the faeces from the intestine into the colder exterior environment. The cysts, however, are well adapted to survival outside the body and may be found viable 66 days after excretion (Brumpt, 1949).

We have observed viable cysts in preparations of faeces in isotonic saline kept on the bench for 6 weeks, but when the same preparations were kept at 37° C. survival was briefer and no viable cysts were found after 6 days.

Cysts in faeces were studied at intervals during

the process of spontaneous degeneration and an increasing proportion was seen to become susceptible to eosin staining; the protoplasm shrank within the walls of some cysts, and finally a number of cysts disintegrated. These changes were accelerated by raising the temperature to 37° C.

When cysts were exposed to the action of deleterious substances in these experiments, the relative severity of damage was assessed by comparing the proportion of cysts visibly altered after exposure to the substances concerned with the proportion which had degenerated spontaneously in preparations of faeces in isotonic saline kept for the same time under the same conditions. Some substances induced different changes from those which appeared spontaneously; for example, in acetic acid preparations the cyst nuclei stained specifically with eosin, and in sodium hydroxide preparations the cyst walls were attacked directly. Throughout the experiments the rate at which cysts degenerated varied with the concentration of the damaging substance, and the proportion of cysts affected rose with the passage of time. Furthermore, some cysts always degenerated hours or days before the remainder in the same preparation. This suggests that there are differences in resistance between apparently similar cysts, even when all are viable as judged by insusceptibility to staining by eosin. It may be that this difference between cysts is related to the variable intensity of iodine staining often seen in faecal preparations, and perhaps to the age of the parasite since encystment. There may also be differences in the permeability of the walls of cysts.

The cyst wall was rapidly attacked by sodium hydroxide but was resistant to damage by other substances used in these experiments. When it is desirable to kill cysts rapidly, as in the cleansing of premises, the disinfectant used should have been tested against cysts *in vitro*, for it is possible that a fairly toxic substance may be relatively ineffective, because it is slow to penetrate the cyst wall. In these experiments, Lysol and Dettol in 1 % solution were found to kill 100 % of cysts within 30 min.

(*b*) *Damaging effect of mepacrine*

Mepacrine had a slow progressive effect upon cysts of *Giardia*, first staining the parasite yellow, and then giving rise to protoplasmic shrinkage. The cyst walls appeared to be unaffected. This shrinking of protoplasm was only equalled when cysts were exposed to the surface-tension-lowering action of a strong detergent, and it is improbable that mepacrine acts in the same way. Equivalent changes were not induced by substances influencing the pH and osmotic pressure.

The appearance of cysts exposed to mepacrine *in vitro* was similar to that of the abnormal bodies, now presumed to have been degenerate *Giardia* cysts, which were seen during and shortly after the treatment by mepacrine of patients with *Giardia* infections.

The experimental results can be compared with the findings in the case of Giardiasis in a 4-year-old girl, whose faeces were examined daily during treatment on a high dosage of mepacrine (0·2 gm. daily for 6 days). Among the cysts present, an increasing number showed the 'mepacrine-degeneration', until the 5th day of treatment, but no normal parasites were seen after the 4th day. On a lighter dosage eradication would probably have taken a day or two longer, since it has been shown that both time and concentration are important in the action of mepacrine upon this parasite, especially in its cystic stage.

This may explain why under-treatment, in terms either of dosage or duration, is so often followed by relapse. The recommended dosages are near to the minimum that is effective.

The finding of degenerate cysts several days after the completion of treatment in six cases which did not subsequently relapse suggests that the presence of 'mepacrine-degenerated' cysts in the absence of normal cysts is not necessarily an indication that treatment has failed. Such patients should, nevertheless, have further specimens examined until a sequence of completely normal faeces has been obtained.

(*c*) *Resistance of cysts to acid*

A proportion of *Giardia* cysts appeared to survive for as long as 48 hr. when exposed to the action of hydrochloric acid in the highest concentration found in the normal human stomach. The normal gastric acidity does not, therefore, protect against infection with this parasite.

SUMMARY

1. Mepacrine has a damaging action upon both vegetative forms and cysts of *Giardia intestinalis*. The action upon the former is rapid; but the action upon the cysts is gradual, and it takes 4 days of incubation in a 0·06 % mepacrine suspension before all normal cysts disappear. The degeneration of cysts is accelerated by raising the concentration of mepacrine to which they are exposed.

2. Mepacrine produces a characteristic degeneration of the cyst which can be seen both *in vitro* and *in vivo*. This appearance develops gradually. In affected cysts the wall appears unaltered; but the protoplasm becomes shrunken and yellow, occupying only a small part of the internal cyst space. Whether shrunken or not, protoplasm stained by mepacrine is probably too much injured to survive, although

the intense yellow colour makes it difficult to demonstrate eosin staining.

3. When parasites showing the characteristic abnormality are seen in the faeces they should be recognized as degenerate cysts, and further specimens should be examined before the patient is pronounced free from infection.

4. The usual method of treating a *Giardia* infection with mepacrine in a dosage of 0·05–0·3 gm. daily according to age, given by mouth for 6 days successfully eradicates the parasite, which is killed in both cystic and vegetative stages, but this dose is near to the minimum effective and neither time nor dosage can safely be reduced if a reliable cure is wanted.

5. Caustic soda destroys the whole cyst fairly rapidly, while a detergent had a damaging action on the protoplasm. Alkaline detergent solutions may therefore be recommended for the cleansing of infected utensils and premises. Dettol and Lysol kill cysts within 30 min. (judged by eosin staining), and 1 % solutions of these disinfectants appear as effective as 5 %. Utensils should be left to soak in the disinfecting solution since some cysts are slower to be affected than are others.

6. Cysts of *Giardia* are fairly resistant to acid, and up to 50 % of them survived 4 hr. incubation in hydrochloric acid at a concentration of 0·26 gm.%, which corresponds to the upper limit of normal human gastric acidity (Harrison, 1947).

7. The cysts were damaged in different ways by different substances. In some cases the cyst was unchanged in appearance and yet rendered susceptible to eosin staining, in others the nuclei alone stained with eosin. Sometimes the protoplasm shrank within an intact cyst wall, and sometimes the wall was attacked directly.

8. In each sample of faeces studied in these experiments the *Giardia* cysts present appeared to be of unequal vitality, since, whatever the test substance to which they were exposed, and whatever form of degeneration developed, some cysts were affected hours or days before others showed any changes. It is conjectured that the age of the parasite since encystment may influence its resistance to damage.

Grateful acknowledgements are made to the practitioners and Medical Officers who co-operated in the following-up of cases of Giardiasis after they had been treated by mepacrine.

REFERENCES

Brown, E. H. (1948). *Arch. Dis. Child.* **23**, no. 114, 19.
Brumpt, L. (1937). *C.R. Soc. Biol., Paris*, **124**, 1040.
Brumpt, E. (1949). *Précis de parasitologie*, 6th ed. Paris.
Galli-Valerio, B. (1937). *Schweiz. med. Wschr.* **67**, 1181
Harrison, G. A. (1947). *Chemical Methods in Clinical Medicine*, 3rd ed. London.
Hoare, C. A. (1949). *Handbook of Medical Protozoology.* London.
Ormiston, G., Taylor, J. & Wilson, G. S. (1942). *Brit. med. J.* **2**, 151.
Sudds, M. V. N. (1943). *Mon. Bull. Emerg. Publ. Hlth Serv.* **2**, 53.
Todd, J. C. & Sanford, A. H. (1943). *Clinical Diagnosis by Laboratory Methods*, 10th ed. Philadelphia.

(*MS. received for publication* 21. xi. 1951.—Ed.)

RADIOACTIVE LABELLING EXPERIMENTS WITH *PLASMODIUM*

By S. W. A. KUPER, M.B., B.Ch., D.P.H., D.T.M. & H., D.C.P.
Department of Parasitology, London School of Hygiene and Tropical Medicine
and S. R. PELC, Ph.D.
Radiotherapeutics Research Unit of the Medical Research Council, Hammersmith Hospital

(With Plate V)

In an endeavour to demonstrate early pre-erythrocytic forms of the malarial parasite, a series of experiments was undertaken to investigate the possibilities of incorporating radioactive tracers in the organism. *Plasmodium gallinaceum* was selected as the test parasite because of ready availability of gametocytes, occurrence of cryptozoites at site of inoculation, and ease of rearing and maintaining the insect host—*Aëdes aegypti*.

It was decided to demonstrate the parasite by autoradiography (Doniach & Pelc, 1950) in histological sections from which all tracer present, not linked to protein or deoxyribonucleic acid, is removed by hydrolysis (Howard & Pelc, 1951). Sections were stained by Giemsa-colophonium (Shortt & Cooper, 1948), but as this faded during photographic processing, unstained sections were observed by phase-contrast. By removing the phase plate the autoradiograph only is observed, and the two methods of examination enable one to locate with accuracy any activity present in the tissues. In experiments with mosquitoes described below, all sections were cut serially, Giemsa stains were done on alternate numbers and autoradiographs made of the intervening sections. This enables one to select a stained section of an individual oocyst, and examine the autoradiograph of the *same* oocyst at a slightly different level.

So little is known of metabolic requirements of *Plasmodium* in the definitive host, that the choice of tracer presented some difficulty. Experiments were performed rearing *Aëdes* in radio-active phosphorus solution, and later by feeding normally reared and malaria-infected mosquitoes on radioactive phosphorus or radioactive sulphur.

It was decided to perform the initial experiments with phosphorus (^{32}P) which has been extensively used for 'marking' *Aëdes* (Hassett & Jenkins, 1949, and Bugher & Taylor, 1949). Haas & Wilcox (1950) have demonstrated marked retardation of oocyst development in *Aëdes* fed on glucose solution containing radioactive phosphorus.

In our first experiment, adult *Aëdes* were produced by immersing 3rd instar larvae in water containing 15 μC./ml. of ^{32}P, but autoradiographs of oocysts were unsatisfactory. The half-life of ^{32}P is 14 days, and as a long period must necessarily elapse before such mosquitoes are ready to infect the test birds (with loss of over half the initial radiation), this method was abandoned in favour of feeding ^{32}P to mosquitoes that had already been infected with malaria.

To determine the possible influence of radiation damage, batches of *Aëdes* in three cages were given an infective feed on a suitable bird, and then maintained on sugar solution containing 1, 0·5 and 0·2 mC./ml. of ^{32}P respectively. (To conserve material the feeding containers used were of about 1 ml. capacity, made of glass tubing, sealed at one end, with a wick of lint protruding from the free end. These tubes were refilled daily with distilled water.) It was noted that slight retardation resulted in the 0·2 mC./ml. cage, as compared with a control batch. In the 0·5 and 1·0 mC./ml. cages, this effect was more marked, but from about the 18th day after the infective feed, sporozoites appeared in the salivary glands of a few mosquitoes from each of the 0·5 and 1·0 mC./ml. cages. A chick bitten by four mosquitoes in the 0·2 mC./ml. cage showed schizonts in the peripheral blood after 8 days. A chick bitten by six mosquitoes from the 0·5 mC./ml. cage showed no blood parasites after 9 days. None of the mosquitoes from the 1·0 mC./ml. cage fed on the chick offered.

Autoradiographs and stained sections were made of mosquitoes from the 0·5 mC./ml. cage 11 days after the infective feed. The results are shown in photographs (Pl. V, figs. 1–3). Fig. 1 is a stained section, through midgut, showing four oocysts. Fig. 2 is a phase-contrast photograph of the consecutive section, showing the same oocysts. Fig. 3 is a photograph of the *same* section as fig. 1, using conventional microscopy. It will be noticed that the film of photographic emulsion has slipped

slightly on the slide shown in figs. 2 and 3, so that the autoradiograph of each oocyst, instead of lying immediately above, is displaced slightly to one side.

The next experiment was performed on similar lines but with slight modifications, and it enabled us to repeat the above autoradiographic results with a new batch of mosquitoes fed on 0·5 mC./ml. of ^{32}P. (On this occasion the ^{32}P solution was partly neutralized by the addition of N/5-NaOH to a pH of about 6·5.) Feeds used were 3, 1 and 0·5 mC./ml. During the first week the feeds were refilled with distilled water, but on the 8th day they were replenished by adding more of the original solutions, as about sixty mosquitoes were kept in each cage and a dilution effect of the ^{32}P was feared. Within 2 days, nearly all the mosquitoes fed on 3 mC./ml. had died. From the 7th day onwards, midguts were examined by dissection. Retardation in development of oocysts (as judged by size) was noted in all cages as compared with normal controls. By the 10th day, this effect was striking in the 3 mC./ml. cage, but appeared less marked and approximately equal in the other two cages. On the 11th day after the infective feed, sporozoites were present in the salivary glands of the control group, while none was seen in the few dissections done on mosquitoes from the 0·5 mC./ml. cage until the 17th day—and until the 27th day in the 1 mC./ml. cage.

For the next experiment, a batch of *Aëdes* was reared normally, infected with *P. gallinaceum* and maintained on sugar solution containing radioactive sulphur (^{35}S), in the form of sulphate ion. The half-life of ^{35}S is 87 days so that delays of a few days are less significant from the point of view of autoradiography. ^{35}S, on account of the weaker β-rays emitted, is biologically far less destructive than ^{32}P. It was found that a concentration of 0·5 mC./ml. caused no retardation in development of the parasite, sporozoites appearing in the glands at the same time as the control batch.

For the last experiment, much larger numbers of mosquitoes were used and replenishment of liquid in the feeds was always by distilled water. It is felt that appreciable and progressive dilution of tracer resulted.

In addition to the control cage, three cages were set up as follows:

(1) 400 infected mosquitoes, fed on ^{32}P, 0·4 mC./ml.
(2) 250 infected mosquitoes, fed on ^{35}S, 1·0 mC./ml.
(3) 250 infected mosquitoes, fed on ^{35}S, 3·0 mC./ml.

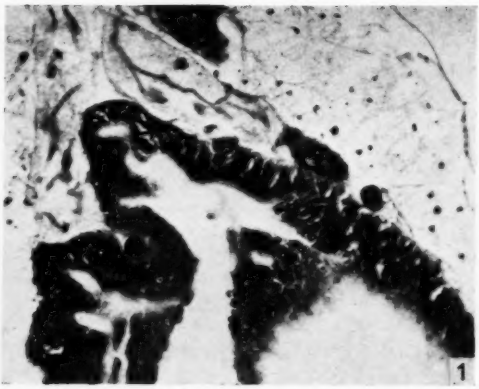
Six days after the infective feed, dissections from each cage showed no retardation in size of oocysts. Ten days after the infective feed, dissection of midguts showed little, if any, reduction in size of oocysts in the 0·4 mC./ml. of ^{32}P cage; results with the two ^{35}S cages were very variable in the few insects dissected. Sections were made from the 3 mC./ml. ^{35}S cage, and the results are shown in photographs (Pl. V, figs. 4–6). Fig. 4 shows a stained section with two oocysts; fig. 5 the consecutive section, shows the same oocysts at a slightly lower level, viewed under phase-contrast; fig. 6, the same section as fig. 5, is a photograph of fig. 5 under the conventional microscope, showing the autoradiograph given by the gut and oocysts. The period of exposure was 5 days, so that the autoradiograph could be greatly enhanced by exposure up to 80 days or longer. It is at least shown that ^{35}S, presented to the mosquito in the form of sulphate ion, is metabolized by the parasite, but whether it be present in the sporozoite or in the oocyst fluid is uncertain. From 10 days after the infective feed, dissections showed sporozoites in the salivary glands in mosquitoes from all the cages.

So far, efforts to produce autoradiographs of sporozoites and cryptozoites have not been successful. In sections of salivary glands containing sporozoites, the activity present in the glands themselves makes it very difficult to assess whether the presence of sporozoites adds to the autoradiograph.

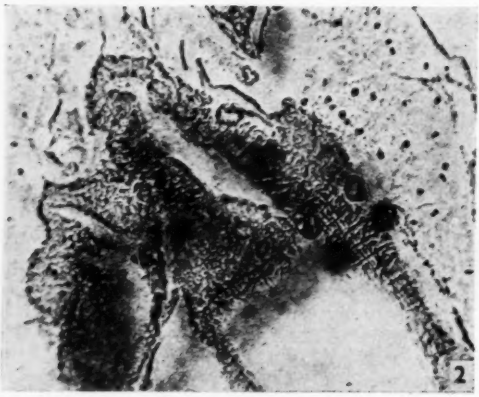
Methionine (synthesized with active sulphur—^{35}S) would seem a suitable substance for further experiments, for McKee & Geiman (1948) have demonstrated that *P. knowlesi* has appreciable need of this amino-acid in the vertebrate host. It is, of course, uncertain whether this be equally true of other species, or whether the needs differ materially in the insect host. It appears, therefore, that active sulphur, used either as sulphate ion or possibly as ^{35}S in a sulphur-containing amino-acid, offers a promising line of investigation.

SUMMARY

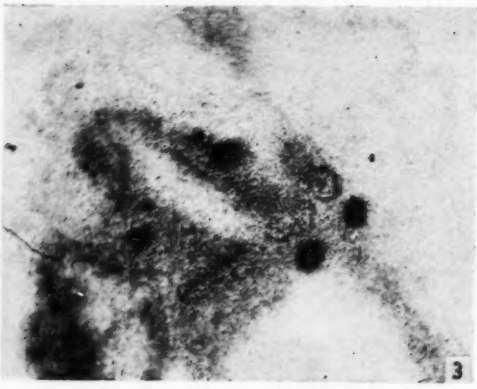
An account is given of the methods used to produce radioactive oocysts of *P. gallinaceum*. The latter are demonstrated in stained sections and in autoradiographs. The photographs show oocysts in mosquitoes fed on solutions of radioactive phosphorus and radioactive sulphur.

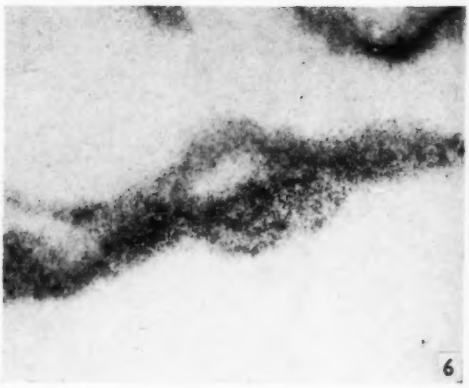

1

4


2

5


3


6

REFERENCES

BUGHER, J. C. & TAYLOR, M. (1949). *Science*, **110**, 146.

DONIACH, I. & PELC, S. R. (1950). *Brit. J. Radiol.* **23**, 184.

GEIMAN, Q. M. (1948). *Proc. IVth Int. Congr. Trop. Med. Mal.* pp. 618–28.

HAHN, P. F., HAAS, V. H. & WILCOX, A. (1950). *Science*, **8**, 657.

HASSETT, C. C. & JENKINS, D. W. (1949). *Science*, **110**, 109.

HOWARD, A. & PELC, S. R. (1951). *Exp. Cell. Res.* **2**, 178.

MCKEE, R. W. & GEIMAN, Q. M. (1948). *Fed. Proc.* **7**, 172.

SHORTT, H. E. & COOPER, W. (1948). *Trans. R. Soc. Trop. Med. & Hyg.* **41**, 427–8.

EXPLANATION OF PLATE V

Figs. 1–3. Serial sections through midgut of *Aëdes* mosquito fed on ^{32}P showing four oocysts of *P. gallinaceum*: fig. 1, stained section; fig. 2, consecutive section viewed by phase-contrast; fig. 3, identical section seen in fig. 2, viewed by conventional microscopy.

Figs. 4–6. Serial sections through midgut of *Aëdes* mosquito fed on ^{35}S, showing two oocysts of *P. gallinaceum*: fig. 4, stained section; fig. 5, consecutive section viewed by phase-contrast; fig. 6, identical section seen in fig. 5, viewed by conventional microscopy.

(*MS. received for publication* 17. VIII. 1951.—Ed.)

THE ACTION OF 2:4-DIAMINO-6:7-DI*ISO*PROPYLPTERIDINE ON NORMAL, PROGUANIL- AND SULPHADIAZINE-RESISTANT STRAINS OF *PLASMODIUM GALLINACEUM*

By ELSPETH W. McCONNACHIE, *Molteno Institute, University of Cambridge*

CONTENTS

	PAGE		PAGE
I. Introduction	272	IV. Discussion	273
II. Methods	272	V. Summary	275
III. Experiments	273	References	276

I. INTRODUCTION

Of six pteridines tested against *Plasmodium gallinaceum* in the chick by Greenberg (1949*a*), only one, 2:4-diamino-6:7-diphenylpteridine, showed antimalarial activity at doses tolerated by the host. More recently, 2:4-diamino-6:7-di(*p*-chlorophenyl)-pteridine and 2:4-diamino-6(or 7)-*o*-chlorophenyl-7 (or 6)-*p*-methoxyphenylpteridine have also been shown to possess antimalarial activity (Falco, Goodwin, Hitchings, Rollo & Russell, 1951). In testing a series of pteridines for vibriostatic activity, several compounds, including 2:4-diamino-6:7-di*iso*-propylpteridine (0/129) and 2:4-diamino-1′-methyl-indolo-(2′:3′-6:7)-pteridine (0/120/II) were found to have marked activity (Collier, Campbell & Fitzgerald, 1950; Collier & Waterhouse 1950*a*). It seemed of interest, therefore, to discover whether these compounds also possess antimalarial activity.

2:4-Diamino-6:7-diphenylpteridine has certain characteristics in common with proguanil (chlorguanide, paludrine). Greenberg, Boyd & Josephson (1948) found that proguanil markedly potentiated the action of sulphadiazine on *P. gallinaceum* in the chick, and Greenberg (1949*c*) was later able to show that the antimalarial action of proguanil is partially antagonized by pteroylglutamic acid. Antagonism between proguanil and pteroylglutamic acid has also been demonstrated in the *Lactobacillus casei* system (Falco, Hitchings, Russell & VanderWerff, 1949). Certain diaminopteridines, which are antagonized by pteroylglutamic acid, have been known for some time to potentiate the antibacterial action of sulphonamides (Daniel & Norris, 1947), and similar phenomena have been demonstrated for the action of 2:4-diamino-6:7-diphenylpteridine against *Plasmodium gallinaceum* (Greenberg, 1949*a*). Further evidence of a relationship in mode of antimalarial action of this pteridine and proguanil was provided by the observation that a strain of *P. gallinaceum* resistant to proguanil was also resistant to 2:4-diamino-6:7-diphenylpteridine (Greenberg & Richeson, 1951).

Resistance to proguanil does not, however, confer resistance to all compounds which potentiate sulphadiazine and are antagonized by pteroylglutamic acid. Although Bishop & McConnachie (1948, 1950) found cross-resistance to sulphadiazine in one proguanil-resistant strain of *P. gallinaceum* they were unable to obtain it in a second strain. A proguanil-resistant strain of *P. gallinaceum* tested by Greenberg & Richeson (1951) showed no cross-resistance to 2:4-diamino-5-(*p*-chlorophenoxy)-6-methylpyrimidine, although the antimalarial activity of this compound is potentiated by sulphadiazine, and partially antagonized by pteroylglutamic acid (Greenberg & Richeson, 1950). Similar results were obtained by Rollo (1951), when 2:4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine was tested against proguanil-resistant strains of *P. gallinaceum* and *P. berghei*. Bishop & McConnachie (1948, 1950) found that a sulphadiazine-resistant strain of *P. gallinaceum* was highly resistant to proguanil, though at no time in its history had it been in contact with this drug. It was thought that tests between active pteridines and proguanil- and sulphadiazine-proguanil-resistant strains of *P. gallinaceum* might throw further light upon the relationship of these compounds.

I am indebted to Dr H. O. J. Collier and Messrs Allen and Hanbury for supplies of 0/129, 0/120/II and 2:4-diamino-6:7-diphenylpteridine, described by Collier & Waterhouse under the serial number 0/63.

II. METHODS

The antimalarial activity of the two new pteridine compounds, 0/129 (2:4-diamino-6:7-di*iso*propylpteridine) and 0/120/II (2:4-diamino-1′-methylindolo-(2′:3′-6:7)-pteridine) was tested upon *P. gallinaceum* by the method described by Curd, Davey & Rose (1945), with the exception that the inoculum

consisted of 50,000,000 parasites per bird. The pteridines were administered as suspensions of 0/129 base and 0/120/II phosphate in 10% gum acacia. Compound 0/129 was given orally by catheter tube into the stomach, but 0/120/II was given intraperitoneally, at the suggestion of Dr Collier who found that this compound was not well absorbed when given orally to rats and mice. Proguanil was given as a solution of the hydrochloride, and sulphadiazine as a suspension in 10% gum acacia.

The strains of *P. gallinaceum* used in the present experiments have been maintained in a patent state in chicks 45–65 g. in weight, by intravenous injection of infected blood at intervals of 2 or 4 days. The parasitized blood was mixed with a little heparin-saline to prevent clotting. The normal parent strain has at no time been in contact with any drug, and the drug-resistant strains were developed from it by treating substrains with gradually increasing doses of the drug concerned. The proguanil-resistant strain used for the cross-resistance tests was the second proguanil-resistant strain isolated by Bishop & McConnachie (1950), and shows complete resistance to the maximum tolerated dose of proguanil (1·5 mg.*), and normal sensitivity to sulphadiazine. The sulphadiazine-proguanil-resistant strain, which has been treated only with sulphadiazine (strain D of Bishop & McConnachie, 1950), is now resistant to 2·5 mg. doses of sulphadiazine, and to 1·5 mg. doses of proguanil, though in birds treated with these doses and even in untreated birds its growth is less good than that of the normal strain.

The response of these strains to the pteridines was tested according to the method of Bishop & Birkett (1948). Groups of three to six chicks were inoculated intravenously with 50,000,000 parasites of the drug-resistant strain per bird. One group remained untreated, as controls, while the other groups were treated with the drugs to be tested. The first dose of drug was given immediately before inoculation, and two doses on each of the following 3 days. The intensity of infections produced in each group of chicks was determined on the 4th day after inoculation and compared with the infections produced in similarly treated groups of birds inoculated with approximately 50,000,000 parasites of the normal strain per bird.

III. EXPERIMENTS

The maximum tolerated dose of 0/129 when given twice daily for 3½ days to chicks 45–65 g. in weight was 2·5 mg. In the majority of birds, doses of 0·063 mg. when given over the same period, reduced the growth of the normal strain to a low level (Table 1). The chemotherapeutic index of 0/129 is thus of the order of 1 : 40, approximately three times as great as that of quinine when tested by the same method.

Table 1. *The effect of* 2:4-*diamino*-6:7-*diisopropyl-pteridine* (0/129) *on the normal strain of* Plasmodium gallinaceum

		0/129 in mg./20 g.		
	Controls	0·025	0·063	0·125
Exp. I	391	403	10	8
Exp. II	294	205	24	0

Intensity of infection expressed as the average number of parasitized erythrocytes per 500 erythrocytes, in groups of three to five birds.

Pteridine 0/120/II in doses of 10 mg. was lethal to three out of five birds, and this dose failed to inhibit the growth of parasites. It was therefore without action upon *P. gallinaceum* in doses tolerated by the chick.

The minimum effective dose—i.e. the dose producing a 90% reduction in parasitaemia compared with untreated controls—for 0/63 was found to be 0·64 mg. This differs from the value obtained by Greenberg (1949*a*), but the method used in the present experiments for determining antimalarial activity differed in some details from that employed by him. The proguanil-resistant strain of *P. gallinaceum* was tested against 0·64 and 1·28 mg.; the strain grew well in birds receiving either dose (Table 2). Having thus confirmed Greenberg's observation that a proguanil-resistant strain of *P. gallinaceum* was cross-resistant to 0/63, the reaction of the sulphadiazine-proguanil-resistant strain of *P. gallinaceum* was also tested; it likewise proved to be resistant to 0/63 (Table 2).

When the two resistant strains of *P. gallinaceum* were tested against 0/129, however, no cross-resistance could be demonstrated; indeed, both strains were slightly hypersensitive to this drug (Table 3). The minimum effective dose for the normal parent strain was 0·063 mg., while 0·025 mg. permitted of some growth. The growth of the proguanil- and sulphadiazine-proguanil-resistant strains, on the other hand, was inhibited by 0·025 mg., and to a lesser degree by 0·013 mg., a dose which had no effect on the normal strain.

IV. DISCUSSION

Four pteridines have been shown to possess antimalarial activity. With the exception of 0/129, these active pteridines are 2:4-diamino-6:7-diarylpteridines (Greenberg, 1949*a*; Falco *et al.* 1951).

* Throughout this paper, doses of drug are expressed in mg./20 g. of body weight of bird and were given twice daily.

0/129, a dialkylpteridine, is several times more active than the diarylpteridines. There appears to be no correlation between vibriostatic and antimalarial activity—for example, 0/63, an active antimalarial, shows no action against *Vibrio cholerae*, while 0/120/II, which is very active against *V. cholerae*, is not inhibitory to *Plasmodium*

Structurally, 0/129 differs from 0/63 only in having alkyl groups substituted in the 6, 7 positions of the molecule, where 0/63 has aryl groups. Since these two pteridines have the same basic structure, it might be expected that they would have a common mode of action, owing to their structural similarity to the pteridine part of the folic acid

Table 2. *The effect of* 2:4-*diamino*-6:7-*diphenylpteridine* (0/63) *on proguanil- and sulphadiazine-proguanil-resistant strains of* Plasmodium gallinaceum

		Proguanil in mg./20 g.		Sulphadiazine in mg./20 g.			0/63 in mg./20 g.		
Strain	Controls	0·1	1·0	0·625	1·25	2·5	0·32	0·64	1·28
Proguanil-resistant	393	—	398	—	4	—	—	408	381
Normal	396	—	1	—	1	—	—	4	—
Proguanil-resistant	266	—	281	—	0	—	—	261	—
Normal	291	—	0	—	2	—	—	1	—
Sulphadiazine-resistant	229	174	—	125	90	99	201	114	—
Normal	380	0	—	36	13	—	58	4	—
Sulphadiazine-resistant	223	205	200	—	200	—	141	168	242
Normal	357	0	—	—	4	—	232	6	—

Intensity of infection expressed as the average number of parasitized erythrocytes per 500 erythrocytes, in groups of three to five birds.

Table 3. *The effect of* 2:4-*diamino*-6:7-*diisopropylpteridine* (0/129) *on proguanil- and sulphadiazine-proguanil-resistant strains of* Plasmodium gallinaceum

		Proguanil in mg./20 g.		Sulphadiazine in mg./20 g.		0/129 in mg./20 g.			
Strain	Controls	0·1	1·0	1·25	2·5	0·013	0·025	0·063	0·125
Proguanil-resistant	266	—	281	0	—	3	5	—	0
Normal	291	—	0	2	—	146	141	—	0
Proguanil-resistant	358	—	366	1	—	106	32	—	0
Normal	355	—	1	7	—	417	264	—	3
Sulphadiazine-resistant	186	170	—	112	130	46	5	0	0
Normal	339	0	—	12	—	—	203	69*	1

Intensity of infection expressed as the average number of parasitized erythrocytes per 500 erythrocytes, in groups of three to five birds.

* High value due to abnormally high count in one bird, which presumably failed to absorb the drug.

gallinaceum in doses tolerated by the chick. It is of interest, however, that Collier, Hall & Waterhouse (1950) found appreciable vibriostatic activity among several 2:4-diamino-6:7-dialkylpteridines, but very little in those where the aliphatic chain was less than 2 or more than 3 carbon atoms in length. Of the two dialkylpteridines tested against *P. gallinaceum*, 2:4-diamino-6:7-di*iso*propylpteridine (0/129) shows high activity, and 2:4-diamino-6:7-dimethylpteridine shows none (Greenberg, 1949*a*). Possibly, antimalarials of still greater activity are to be found among the pteridines, and if these act similarly to 0/129, they might be of value in the treatment of proguanil-resistant strains of human malaria.

molecule (Daniel, Norris, Scott & Heuser, 1947). The results obtained in the present experiments, however, show that the mode of action of 0/129 and 0/63 upon *P. gallinaceum* cannot be identical, since the proguanil- and sulphadiazine-proguanil-resistant strains of this parasite are cross-resistant to 0/63 but not to 0/129.

Greenberg (1949*a*) states that preliminary experiments indicate that 0/63 is not active *in vitro* against erythrocytic parasites, and it is possible that, like proguanil, this drug owes its antimalarial properties to the formation *in vivo* of an active compound. It has recently been shown that proguanil is metabolized in the mammalian body to 2:4-diamino-1-*p*-chlorophenyl-1:6-dihydro-

6:6-dimethyl-1:3:5-triazine, which is 10 times as active as proguanil against *P. gallinaceum* in the chick (Carrington, Crowther, Davey, Levi & Rose, 1951). As yet, nothing is known of the mode of action of 0/129 on the malarial parasite; it may act directly, or be 'activated' in the body. Since, however, Collier & Waterhouse (1950*a*) found that 0/63 had no *in vitro* action against *Vibrio cholerae*, unlike 0/129, which was highly active *in vitro*, it may be that 0/63 owes its *in vivo* activity against *P. gallinaceum* to the formation of a metabolite, while 0/129 acts directly on the parasite.

Greenberg & Richeson (1951) conclude that it is probable that the pteridines and proguanil act on the malaria parasite in the same way. The results of the present experiments, however, show that all the pteridines do not act in the same way as proguanil, but that the 2:4-diamino-pteridines form a heterogeneous group of compounds. At the moment it is not known whether the antimalarial action of 0/129 is antagonized by pteroylglutamic acid, or whether 0/129 synergizes with the sulphonamides, though both these phenomena occur against *Vibrio cholerae in vitro* (Collier, Hall & Waterhouse, 1950; Collier & Waterhouse, 1950*b*).

It is unlikely that the inhibitory action of 0/129 is brought about in the same way as that of those pyrimidines to which proguanil-resistant strains of malaria are sensitive; Rollo's resistant strains showed no hypersensitivity to the pyrimidine he tested, whereas the resistant strains of *Plasmodium gallinaceum* used in the present experiments showed a slight but definite hypersensitivity to 0/129. Greenberg (1949*b*) reported that his proguanil-resistant strain of *P. gallinaceum* was hypersensitive to sulphadiazine. If strains of *P. gallinaceum* resistant to 0/129 can be obtained, it will be of interest to determine if they are also resistant to proguanil.

These experiments show that the sulphadiazine-proguanil- and proguanil-resistant strains of *P. gallinaceum* respond in an identical manner to the pteridines tested. Similarly, Rollo (1951) found that sulphadiazine- and proguanil-resistant strains of *P. berghei* showed an identical response to a 2:4-diamino-pyrimidine. It is probable, therefore, that resistance to proguanil, whether induced by sulphadiazine or proguanil, is due to the same cellular mechanism.

V. SUMMARY

1. 2:4-diamino-6:7-di*iso*propylpteridine has an antimalarial index of 1:40, when tested against *Plasmodium gallinaceum*.

2. 2:4-diamino-1′-methylindolo-(2′:3′-6:7)-pteridine showed no activity against *P. gallinaceum* in doses tolerated by the chick.

3. Proguanil- and sulphadiazine-proguanil-resistant strains of *P. gallinaceum* were cross-resistant to 2:4-diamino-6:7-diphenylpteridine.

4. Proguanil- and sulphadiazine-proguanil-resistant strains of *P. gallinaceum* showed no cross-resistance to 2:4-diamino-6:7-di*iso*propylpteridine, but were slightly hypersensitive to this compound.

I wish to thank Dr A. Bishop for her interest in this work, and for advice and criticism. The work has been financed by the Medical Research Council.

REFERENCES

BISHOP, A. & BIRKETT, B. (1948). Drug-resistance in *Plasmodium gallinaceum*, and the persistence of paludrine-resistance after mosquito transmission. *Parasitology*, **39**, 125.

BISHOP, A. & MCCONNACHIE, E. W. (1948). Resistance to sulphadiazine and 'paludrine' in the malaria parasite of the fowl (*Plasmodium gallinaceum*). *Nature, Lond.*, **162**, 541.

BISHOP, A. & MCCONNACHIE, E. W. (1950). Sulphadiazine-resistance in *Plasmodium gallinaceum* and its relation to other antimalarial compounds. *Parasitology*, **40**, 163.

CARRINGTON, H. C., CROWTHER, A. F., DAVEY, D. G., LEVI, A. A. & ROSE, F. L. (1951). A metabolite of 'paludrine' with high antimalarial activity. *Nature, Lond.*, **168**, 1080.

COLLIER, H. O. J. & WATERHOUSE, P. D. (1950*a*). Studies in the chemotherapy of cholera. II. *In vitro* vibrio-static properties of certain 2:4-diamino-pteridines. *Ann. Trop. Med. Parasit.* **44**, 156.

COLLIER, H. O. J. & WATERHOUSE, P. D. (1950*b*). Studies in the chemotherapy of cholera. IV. Antagonism of the antibacterial activities of 2:4-diamino-pteridines, sulphaguanidine, and their mixtures. *Ann. Trop. Med. Parasit.* **44**, 273.

COLLIER, H. O. J., CAMPBELL, N. R. & FITZGERALD, M. E. H. (1953). Vibriostatic activity in certain series of pteridines. *Nature, Lond.*, **165**, 1004.

COLLIER, H. O. J., HALL, I. F. & WATERHOUSE, P. D. (1950). Studies in the chemotherapy of cholera. III. The action of pteridine-sulphonamide mixtures upon *Vibrio cholerae* and upon the mouse. *Ann. Trop. Med. Parasit.* **44**, 161.

CURD, F. H. S., DAVEY, D. G. & ROSE, F. L. (1945). Studies on synthetic antimalarial drugs. I. Biological methods. *Ann. Trop. Med. Parasit.* **39**, 139.

DANIEL, L. J. & NORRIS, L. C. (1947). Growth inhibition of bacteria by synthetic pterins. II. Studies with *Escherichia coli*, *Staphylococcus aureus* and *Lactobacillus arabinosus* showing synergism between pterin and sulfonamide. *J. Biol. Chem.* **170**, 747.

DANIEL, L. J., NORRIS, L. C., SCOTT, M. L. & HEUSER, G. F. (1947). Growth inhibition of bacteria by

synthetic pterins. I. Studies with *Streptococcus faecalis*, *Lactobacillus casei* and *Lactobacillus arabinosus*. *J. Biol. Chem.* **169**, 689.

FALCO, E. A., GOODWIN, L. G., HITCHINGS, G. H., ROLLO, I. M. & RUSSELL, P. B. (1951). 2:4-diaminopyrimidines—a new series of antimalarials. *Brit. J. Pharmacol.* **6**, 185.

FALCO, E. A., HITCHINGS, G. H., RUSSELL, P. B. & VANDERWERFF, H. (1949). Antimalarials as antagonists of purines and pteroylglutamic acid. *Nature, Lond.*, **164**, 107.

GREENBERG, J. (1949*a*). The antimalarial activity of 2, 4-diamino-6, 7-diphenylpterin; its potentiation by sulfadiazine and inhibition by pteroylglutamic acid. *J. Pharmacol.* **97**, 484.

GREENBERG, J. (1949*b*). Hypersensitivity to sulfadiazine of a chlorguanide-resistant strain of *Plasmodium gallinaceum*. *J. Nat. Malaria Soc.* **8**, 80.

GREENBERG, J. (1949*c*). Inhibition of the antimalarial activity of chlorguanide by pteroylglutamic acid. *Proc. Soc. Exp. Biol., N.Y.*, **71**, 306.

GREENBERG, J., BOYD, B. L. & JOSEPHSON, E. S. (1948). Synergistic effect of chlorguanide and sulfadiazine against *Plasmodium gallinaceum* in the chick. *J. Pharmacol.* **94**, 60.

GREENBERG, J. & RICHESON, E. M. (1950). Potentiation of the antimalarial activity of sulfadiazine by 2, 4-diamino-5-aryloxypyrimidines. *J. Pharmacol.* **99**, 444.

GREENBERG, J. & RICHESON, E. M. (1951). Effect of 2, 4-diamino-5-(*p*-chlorophenoxy)-6-methylpyrimidine and 2, 4-diamino-6, 7-diphenylpteridine on chlorguanide-resistant strain of *Plasmodium gallinaceum*. *Proc. Soc. Exp. Biol., N.Y.*, **77**, 174.

ROLLO, I. M. (1951). A 2:4-diaminopyrimidine in the treatment of proguanil-resistant laboratory malarial strains. *Nature, Lond.*, **168**, 332.

(*MS. received for publication* 28. II. 1952.—Ed.)

THE DEVELOPMENT OF RESISTANCE TO METACHLORIDINE IN *PLASMODIUM GALLINACEUM* IN CHICKS

BY ANN BISHOP* AND ELSPETH W. McCONNACHIE
From the Molteno Institute, University of Cambridge

CONTENTS

	PAGE
1. Introduction	277
2. Methods	278
3. The development of resistance to metachloridine in strains of *Plasmodium gallinaceum* maintained in young chicks	279
4. The relationship of metachloridine to other antimalarial compounds as demonstrated by cross-resistance tests	281
5. Discussion	283
6. Summary	284
References	285

1. INTRODUCTION

It was first shown, by Bishop & Birkett (1947) and Williamson, Bertram & Lourie (1947), working independently, that *Plasmodium gallinaceum* can readily be made resistant to the antimalarial compound proguanil (paludrine, N'-*p*-chlorophenyl-N^5-*iso*propylbiguanide) by treating the host with gradually increasing doses of the drug. The ability to become resistant to this drug is not confined to *P. gallinaceum*, as, more recently, proguanil-resistant strains of *P. cynomolgi* (Hawking & Perry, 1948; Schmidt, Genther, Fradkin & Squires, 1949), *P. falciparum* (Adams & Seaton, 1949) and *P. vivax* (Lourie & Seaton, 1949; Cooper, Coatney & Imboden, 1950) have been developed. As with *P. gallinaceum*, these strains were maintained by blood inoculation during the period in which resistance to the drug was developing, and they, like *P. gallinaceum*, retained their resistance after passage through mosquitoes (Schmidt *et al.* 1949; Seaton & Adams, 1949; Seaton & Lourie, 1949).

Reports from Malaya (Field & Edeson, 1949; Edeson & Field, 1950) suggest that proguanil-resistance has developed in the normal course of treatment in a strain of *P. falciparum* in the Tampin district, and according to van Goor, Lodens & Gomez (1950), proguanil-resistant strains of *P. malariae* and *P. vivax* have been observed in a district of Indonesia, where the population has been treated prophylactically with the drug for over a year. These observations suggest that the development of drug-resistance in malaria may prove to be a problem of more than academic interest.

Hitherto, apart from proguanil, drug-resistant strains of malaria parasites have been produced only with pamaquin, quinine and the sulphonamides—sulphadiazine and sulphanilamide. A fourfold increase in resistance to pamaquin was produced in *P. knowlesi* by Fulton & Yorke (1941, 1943); more recently, some resistance to this drug has been developed in a strain of *P. gallinaceum*, but the total enhancement obtained was only four- to eightfold, and its development was slow as compared with that of proguanil-resistance (Bishop & Birkett, 1948; Bishop & McConnachie, 1952*b*). With quinine, also, the increase in resistance in response to the drug was slight, being only twofold (Knoppers, 1947, 1949). Resistance to sulphadiazine, obtained by treating *P. gallinaceum* with that drug, was slow in developing, but a 32-fold increase in resistance was ultimately produced (Bishop & McConnachie, 1950*a*). Resistance to sulphanilamide was obtained in *P. gallinaceum* by treatment with that drug, but as its chemotherapeutic index is 2, the maximum increase in resistance was only twofold (Bishop & McConnachie, 1950*b*).

Attempts to produce resistance to mepacrine and chloroquine in *P. gallinaceum*, *P. lophurae* and *P. cynomolgi* have all failed (Bishop & Birkett, 1947, 1948; Williamson & Lourie, 1947; Thompson, 1948; Schmidt *et al.* 1949; Bishop, 1951; Seaton, 1951; Bishop & McConnachie, 1952*a*), as have the attempts to produce resistance to pentaquine and *iso*pentaquine in *P. cynomolgi* (Schmidt *et al.* 1949).

There is evidence that the modes of antimalarial action of sulphadiazine and proguanil are related. With *P. gallinaceum* sulphadiazine has been shown to produce resistance not only to itself, but to proguanil also (Bishop & McConnachie, 1948, 1950*a*), an observation which has been confirmed by Rollo (1951) on *P. berghei*. Although these observations suggest that the modes of action of proguanil and sulphadiazine are related, they cannot be identical,

* Member of the Scientific Staff of the Medical Research Council.

as the antimalarial action of proguanil is not, like that of sulphadiazine, antagonized by *p*-aminobenzoic acid (PABA). Greenberg (1949*a*), however, has found that pteroylglutamic acid, which antagonizes the antimalarial action of sulphadiazine completely, is able to antagonize that of proguanil partially, but the experimental data suggest that this partial antagonism, unlike the complete antagonism produced by PABA upon sulphadiazine, is not competitive. Previously, evidence of a relationship between sulphadiazine and proguanil had been produced by Greenberg, Boyd & Josephson (1948), who found that the antimalarial action of these drugs is potentiated if they are given drug-diet technique) did not alter the normal food intake or the growth of the birds. Such doses gave a maximum blood level of 10 mg./100 ml. of blood. With higher doses of the drug, giving a maximum blood level of 30 mg./100 ml., loss in weight occurred.

According to Brackett & Waletzky the antimalarial action of metachloridine, unlike that of sulphanilamide, sulphadiazine and related sulphonamides, is not antagonized by PABA, an observation which we have confirmed upon our strain of *P. gallinaceum*, nor is its action antagonized by pteroylglutamic acid (Folvite, Lederle) (Table 1). Moreover, the metanilamides, according to Brackett & Waletzky, are inactive or only slightly active

Table 1. *The antimalarial activity of metachloridine upon the parent strain of* Plasmodium gallinaceum *and the effect of* p-*aminobenzoic acid* (*PABA*) *and pteroylglutamic acid* (*PGA*) *upon its action*

	Metachloridine*				PABA*		PABA*+metachloridine		PGA*		PGA*+metachloridine			
Control	0·2	0·1	0·05	0·025	0·2	0·1	0·2+0·2	0·1+0·1	0·8	0·2	0·8+0·2	0·2+0·2	0·8+0·05	0·2+0·05
384	—	—	35	—	—	—	—	—	—	—	—	—	—	—
254	—	10	15	121	—	274	—	20	—	—	—	—	—	—
256	3	—	—	—	148	—	3	—	282	221	7	6	—	—
323	—	—	44	—	—	—	—	—	—	—	—	—	54	60

* Throughout the tables, the dose, given twice daily, is expressed in mg./20 g. of body weight of bird. The intensity of infection is expressed as the average number of infected erythrocytes per 500 erythrocytes in groups of four to six birds.

concurrently, and this synergistic effect has been found to extend to PABA-competitors other than sulphadiazine, provided that they themselves possess antimalarial activity (Greenberg, 1949*b*). It has been suggested by Beerstecher & Shive (1947), from their experience with bacteria, that synergism usually results from the prevention, by two inhibitors, of two different enzymes in one series of enzymic reactions.

As the only two antimalarial compounds to which a high degree of resistance has been developed have thus been shown to bear some relationship in their modes of action, it was of interest to discover whether resistance could develop readily to some unrelated compound. In choosing a compound it was essential that its chemotherapeutic index, when tested against *P. gallinaceum*, should be large so as to make possible the development of a high degree of resistance to the drug.

Metachloridine, SN 11,347 (3-metanilamido-5-chloropyrimidine), a derivative of metanilamide, an isomer of sulphanilamide, has been shown by Brackett & Waletzky (1946) to be at least 16 times as active upon *P. gallinaceum* as sulphadiazine or quinine on a dose basis, and 6 times as active as sulphadiazine on the basis of the blood concentration. It is extremely well tolerated by chicks; doses of 15 mg./20 g. per day (administered by the

against a number of organisms sensitive to the sulphonamides. We therefore chose metachloridine for further experiments upon the production of drug-resistance in *P. gallinaceum*.

2. METHODS

The strain of *P. gallinaceum* used in these experiments was the normal parent strain from which the resistant strains described in previous papers have been derived (Bishop & Birkett, 1947, 1948; Bishop & McConnachie, 1948, 1950*a*, *b*). With the exception of short periods, when it has been passaged by intramuscular injection, the strain has been maintained by intravenous injection at intervals of 2–3 days for over 4 years. Its sensitivity to the antimalarials with which we have been working has been tested frequently and has remained unchanged. The substrains undergoing treatment with metachloridine have been maintained in the same way, by intravenous injection of heparinized, parasitized blood. Chicks weighing 45–65 g. have been used for maintaining the strains and in the tests for drug-resistance.

The drugs were given once or twice daily, orally, by catheter tube into the stomach, the dose being proportional to the weight of the bird. Metachloridine is insoluble in water, but dissolves

readily if a small quantity of sodium hydroxide is added (Hughes & Brackett, 1946). Sulphadiazine was administered as a suspension in 10% gum acacia. The dose of drug is expressed in mg./20 g. of body weight.

As the dosage used by Brackett & Waletzky was based upon the drug-diet method of administration, it was necessary to determine the maximum tolerated and minimum effective doses of metachloridine when given twice daily in solution. The low toxicity of metachloridine to chicks, described by Brackett & Waletzky, was confirmed. Single doses as large as 50 mg. produced no definite symptoms of toxicity in some chicks, but in others symptoms of acute toxicity occurred within 60 min. Birds which had appeared to be unaffected by a single dose of the drug, however, showed symptoms of toxicity after a second or third dose. Doses of 3·2 mg., which, owing to the limited amount of drug at our disposal, was the largest dose used in developing metachloridine-resistant strains of *P. gallinaceum*, gave rise to no toxic symptoms or loss in weight even when given twice daily for several days. The range of doses of metachloridine which produced partial inhibition of the growth of *P. gallinaceum* was somewhat wide; thus doses of 3·2 mg./20 g., when given twice daily, did not suppress the growth of parasites completely, whereas 0·1–0·05 mg. doses reduced their number to a low level. With still lower doses the proportion of parasitized erythrocytes rose steeply. The minimum effective dose, when given orally, twice daily for 3½ days, in solution, is therefore of the order of 0·1–0·05 mg./20 g.

Tests for drug-resistance were made by the method described previously (Bishop & Birkett, 1947, 1948). When the metachloridine-treated strain was proved to have become resistant to the drug, it was tested for cross-resistance to other antimalarial compounds by the same method.

3. THE DEVELOPMENT OF RESISTANCE TO METACHLORIDINE IN STRAINS OF *PLASMODIUM GALLINACEUM* MAINTAINED IN YOUNG CHICKS

In attempting to produce a strain of *P. gallinaceum* resistant to metachloridine the parasite was passaged, through chicks at intervals of 2–3 days, by intravenous injection of 0·1–1·0 ml. of heparinized blood, the volume depending upon the intensity of infection of the donor bird. It is important in such experiments that the initial doses of the drug should not be large enough to inhibit the growth of the parasite drastically and so convert an overt infection into a latent one, and that, if resistance should develop, increase in dosage should not outstrip its development. The dose of metachloridine administered in the first instance was 0·025 mg.

Table 2. *Comparison of the effect of metachloridine upon a metachloridine-treated strain of* Plasmodium gallinaceum *and the untreated parent strain*

Strain	No. of passages through treated birds	Control	Metachloridine 3·2	0·8	0·4	0·2	0·05
Metachloridine	22	231	151	267	—	319	287
Parent	—	405	—	—	—	37	35
Metachloridine	27	127	57	87	143	161	—
Parent	—	339	—	5	5	4	—
Metachloridine	32	211	184	143	—	283	266
Parent	—	299	—	15	—	15	49
Metachloridine	48	330	318	—	—	—	—
Parent	—	289	4	—	—	—	—
Metachloridine	61	302	399	—	—	—	—
Parent	—	404	12	—	—	—	—

once daily, but as this had no apparent effect upon the parasites or their growth rate, it was given twice daily. In birds receiving the twice-daily doses abnormal parasites were seen in the first two passages, but in later passages parasites were normal in appearance. The dose was gradually increased at intervals of 6–7 days, but these higher doses apparently produced no adverse effect on the parasites. After 9 weeks' treatment and 22 passages through treated birds, doses of 3·2 mg. were being given twice daily without any apparent effect upon the parasites; indeed, in birds receiving this dosage and inoculated with 0·1 c.c. of heavily infected blood, heavy infections of parasites normal in appearance developed in the course of 2–3 days.

The reactions of the metachloridine-treated strain and of the normal parent strain to doses of metachloridine ranging from 0·05 to 3·2 mg. twice daily were tested by the method described previously (Bishop & Birkett, 1948). In birds inoculated with the metachloridine strain and treated with the drug

the parasites grew well, the infections in birds receiving the lower doses of drug being as heavy as in the untreated controls, though in birds receiving 3·2 mg. they were lighter than those in the controls. The infections produced by the metachloridine strain in the untreated control birds were, however, on the whole less heavy than those produced by the parent strain. The development of the parent strain was suppressed almost completely by the range of doses tested (Table 2).

As resistance to metachloridine developed relatively rapidly, and as the parasites appeared to be affected by the drug only in the first few passages through birds receiving the lowest dose of drug (0·025 mg.), it seemed possible that resistance might develop by the selection of resistant individuals present in the normal population exposed to the action of the drug. It had been observed in birds inoculated with the parent strain and treated with doses such as 0·4 and 0·8 mg., that a few parasites, apparently little affected by the drug, remained in the peripheral blood at the end of the 3½ days' treatment. This suggested that a considerable variation in response to the drug might exist between individual parasites. If the survival of parasites at the end of 3½ days' treatment with the drug were due to an inherent insensitivity to its action, a resistant strain would develop rapidly as a result of the selection and multiplication of insensitive individuals.

To test this possibility a chick was inoculated with 50,000,000 parasites of the untreated parent strain and treated twice daily with doses of 0·8 mg. of metachloridine for 3½ days. On the 4th day a few parasites remained in the peripheral blood, and 1 c.c. of the blood was inoculated into an untreated chick. The parasites multiplied rapidly, and in a few days a heavy infection was produced. These parasites were tested for resistance to doses of 0·05, 0·1 and 0·2 mg. of metachloridine, but they proved to be as sensitive as the parent strain. The rapid development of a metachloridine-resistant strain of *P. gallinaceum*, therefore, was not due to the presence of a high proportion of individuals inherently insensitive to metachloridine.

The early stages in the development of resistance to metachloridine were studied further in a substrain (strain C) of the parent strain which was passaged at intervals of approximately 4 days through birds receiving twice-daily doses of 0·025 mg. of metachloridine. Although parasites were numerous in the first two serial infections, some appeared to be abnormal; in all subsequent infections, however, their appearance was normal and infections were heavy. After the strain had been passaged serially three times through chicks treated with 0·025 mg. doses of the drug, two chicks were inoculated with some of the parasitized blood and treated with 0·5 mg. doses of metachloridine twice daily; the infections produced were slight, though the majority of the parasites were normal in appearance. After a period of 42 days, during which the strain was passaged through birds receiving only 0·025 doses of metachloridine twice daily, its reaction to doses of 0·5 mg. was again tested in the same way; heavy infections of parasites normal in appearance were produced.

These results suggested that the strain had become resistant to a greater concentration of metachloridine than that with which it had been in contact. In these rough tests, however, the number of parasites introduced into each bird was very large but not determined (0·25 c.c. of heavily parasitized blood). Further tests, made from the 19th to 75th passage through birds treated with 0·025 mg. of the drug, by the method usually employed for estimating the intensity of resistance (Bishop & Birkett, 1948), were therefore made in order to assess with greater accuracy the reaction of the strain to higher doses of the drug. With inocula of 50,000,000 parasites the infections produced by strain C, in birds treated with 0·025 mg. of the drug once daily for 3½ days, were equal in intensity to those produced in untreated control birds, but in birds receiving higher doses the infections diminished in intensity with the increase in dosage (Table 3). In birds receiving 0·25 or 0·5 mg. of drug twice daily infections were moderately heavy at the end of the 3½ days' treatment, but in birds receiving 1·0 mg. they were very slight.

Although the strain was maintained in birds treated with twice-daily doses of 0·025 mg. of metachloridine for over 11 months (75 passages) no further significant increase in resistance occurred, but when it was passaged through birds treated with 0·5 mg. doses of the drug a further increase in resistance was observed. After being maintained in birds treated with 0·5 mg. of metachloridine for periods of 4–9 weeks the strain was tested and found to produce heavier infections in birds treated with 1·0 mg. of the drug than formerly (Table 3, strain D).

A further strain, strain E, was isolated from the normal parent strain and maintained in a patent state by intravenous injection at frequent intervals through birds treated with 0·025 mg. doses of the drug twice daily. When the strain had been maintained in this way for 15 days (4 passages) its growth in birds treated with 0·025 mg. of metachloridine twice daily and higher doses of the drug was determined (Table 3). Moderately heavy infections were produced in birds treated with 0·025 mg. of the drug, but in birds receiving higher doses the infections were no heavier than those produced by the parent strain in similar birds. After passage for a further period of 35 days (14th passage) through birds treated with 0·025 mg.

Table 3. *Comparison of the sensitivity to metachloridine of the untreated parent strain of* Plasmodium gallinaceum *and strains C and E treated with 0·025 mg. doses of the drug, and strain D treated with 0·025 and 0·5 mg.*

Strain	No. of passages through treated birds	Control	Metachloridine (dose twice daily in mg. per 20 g. of body weight) 1·0	0·5	0·25	0·1	0·025
Metachloridine C	19	301	—	80	—	351	408
Parent	—	321	—	4	—	13	30
Metachloridine C	22	379	42	39	199	210	—
Parent	—	240	—	6	4	7	—
Metachloridine C	31	416	10	63	172	333	302
Parent	—	443	—	—	—	8	51
Metachloridine C	56	378	31	127	211	336	394
Parent	—	338	—	—	—	12	71
Metachloridine C	67	215	35	223	109	257	295
Parent	—	337	—	—	—	7	76
Metachloridine C	75	392	30	114	187	312	—
Parent	—	423	—	—	—	—	41
Metachloridine D	66	389	110	230	260	316	—
Parent	—	337	—	—	—	7	—
Metachloridine D	73	235	131	135	172	—	—
Parent	—	423	—	—	—	—	41
Metachloridine E	4	365	—	7	13	10	206
Parent	—	231	—	—	—	8	40
Metachloridine E	14	321	13	18	29	89	326
Parent	—	277	—	—	—	16	68
Metachloridine E	24	316	9	20	98	197	256
Parent	—	379	—	—	—	3	17
Metachloridine E	40	403	28	96	100	251	378
Parent	—	422	—	—	—	14	75

of metachloridine the growth of the strain in birds receiving higher doses of the drug was again assessed. The infections produced in birds treated with 0·1 mg. of metachloridine were definitely heavier than in the previous test. A further increase in resistance was observed after the strain had been passaged through birds treated with 0·025 mg. of the drug for a further period of 63 days (24th passage). Finally, after treatment with 0·025 mg. of metachloridine for a total period of between 6 and 7 months (40th passage), infections of parasites normal in appearance were produced in birds treated with 0·5 mg. of the drug, though they were much lighter than those in birds treated with 0·1 or 0·025 mg. doses. In birds receiving the higher doses the intensity of infection varied considerably from bird to bird, whereas in those receiving the lower doses the variability was slight.

Resistance to metachloridine, therefore, gradually increased, although the dose of drug to which the strain of *P. gallinaceum* was exposed remained the same; but in both strains treated throughout with 0·025 mg. doses of the drug resistance reached a maximum. Further increase in resistance was obtained in the first strain, but only after exposure to higher doses of the drug.

4. THE RELATIONSHIP OF METACHLORIDINE TO OTHER ANTIMALARIAL COMPOUNDS AS DEMONSTRATED BY CROSS-RESISTANCE TESTS

It has been shown that strains of *P. gallinaceum* (Bishop & McConnachie, 1948, 1950*a*) and *P. berghei* (Rollo, 1951) made resistant to sulphadiazine by treatment with that drug are thereby made resistant also to proguanil. A study of cross-resistance between a drug-resistant strain of organism and compounds known to be effective upon the parent strain may thus reveal unsuspected relationships in modes of action of drugs.

In studying the relationship of metachloridine to other antimalarial compounds the strain of *P. gallinaceum* which has been made resistant to 3·2 mg. doses of metachloridine was used. Against low but effective doses of pamaquin (0·04 mg. twice daily), mepacrine (0·64 mg. twice daily) and quinine (0·8 mg. twice daily) no significant difference in sensitivity between the metachloridine-resistant and the untreated parent strain was revealed. In both strains either no parasites, or only a few abnormal in appearance,

were found in the peripheral blood on the fourth day following inoculation, though in the untreated control birds the strains grew well. Resistance to metachloridine, therefore, does not confer resistance to these compounds.

The metachloridine-resistant strain was also tested for resistance to the *p*-aminobenzene-sulphonamides sulphadiazine, sulphanilamide, sulphathiazole and sulphapyridine (Table 4). At the time when it was first tested against sulphadiazine the strain was in its 26th passage through metachloridine-treated birds and was already resistant to 3·2 mg. doses of the drug, though as yet its growth in birds receiving this dose was not heavy (Table 2). No resistance to 1·25 mg. doses of sulphadiazine was detected. A month later (31st passage) the strain was tested against 0·625 and 0·312 mg. doses of sulphadiazine; these doses inhibit the growth of the parent strain in the majority of chicks, though the degree of inhibition in those treated with 0·312 mg. is variable. The growth of the metachloridine-strain in birds receiving these doses was definitely heavier than that of the untreated parent strain, though the intensity of infection

Table 4. *Comparison of the reaction of the metachloridine-resistant and normal strains of* Plasmodium gallinaceum *to sulphonamides and proguanil*

Strain	No. of passages through treated birds	Control	Sulphadiazine		
			1·25	0·625	0·312
Metachloridine	26	223	2	—	—
Parent	—	257	3	—	—
Metachloridine	31	274	—	75	197
Parent	—	304	—	2	6
Metachloridine	33	339	36	126	251
Parent	—	368	4	3	18
Metachloridine	48	330	86	192	284
Parent	—	289	4	6	33
			Sulphanilamide		
			4	2	1
Metachloridine	27	250	0	—	—
Parent	—	327	5	—	—
Metachloridine	31	274	—	48	127
Parent	—	304	—	24	197
			Sulphathiazole		
			10	5	2·5
Metachloridine	34	306	—	43	186
Parent	—	240	—	6	9
Metachloridine	61	302	68	245	293
Parent	—	401	—	35	106
			Sulphapyridine		
			10	5	2·5
Metachloridine	34	306	21	123	—
Parent	—	240	8	27	—
Metachloridine	61	302	89	204	331
Parent	—	401	—	41	118
			Proguanil		
			0·1	0·05	
Metachloridine	26	223	1	—	—
Parent	—	257	0	—	—
Metachloridine	27	250	0	—	—
Parent	—	327	1	—	—
Metachloridine	32	211	—	1	—
Parent	—	299	—	15	—

in birds receiving 0·625 mg. doses was variable (Table 4). Similar results were obtained when the strain was tested again after treatment with 3·2 mg. doses of metachloridine for a further period of 2 weeks (33rd passage), but after 4 months' treatment (48th passage) the growth of the strain, both in birds receiving 3·2 mg. doses of metachloridine (Table 2) and in birds treated with sulphadiazine (1·25, 0·625 and 0·312 mg., Table 4), was found to have improved. During the period when the strain was undergoing tests for resistance to sulphadiazine its reaction to sulphanilamide and proguanil was tested, but no resistance to these drugs could be detected (Table 4). Some resistance to sulphathiazole and sulphapyridine was observed.

Three drug-resistant strains of *P. gallinaceum* were tested for cross-resistance to metachloridine. All three strains were resistant to proguanil, but the methods whereby this resistance had been produced differed. One strain had been treated with sulphanilamide and had thereby been made resistant not only to that drug itself, but also to proguanil, and, to a lesser extent, to sulphadiazine. A second strain had been treated with sulphadiazine and was resistant not only to 1·25 mg. doses of that drug and to the low range of doses of sulphanilamide tolerated by chicks, but was also fully resistant to proguanil. The third strain had been treated with proguanil and was fully resistant to that drug, but showed no resistance to sulphadiazine or sulphanilamide. None of the strains, when tested against metachloridine, showed cross-resistance to it (Table 5).

Table 5. *Tests for cross-resistance to metachloridine with sulphanilamide-, sulphadiazine- and proguanil-resistant strains of* Plasmodium gallinaceum

		Metachloridine	
Strain	Control	0·1	0·5
Sulphanilamide	286	14	32
Parent	297	19	42
Sulphadiazine	211	—	19
Parent	311	—	29
Proguanil	342	—	21
Parent	323	—	37

5. DISCUSSION

The experiments described earlier in this paper show that resistance to metachloridine can be developed very readily in acute infections of *P. gallinaceum* in young chicks, an increase in resistance of more than 100-fold being obtained in a comparatively short period. Indeed, resistance developed so readily, and the parasites appeared to be so little affected by the drug, that it was at first suspected that it might arise in a single step, as had been demonstrated for streptomycin-resistance in bacteria.

It has been shown that variants possessing low or high grade resistance to streptomycin may occur in the same bacterial population (Klein & Kimelman, 1946; Alexander & Leidy, 1947; Silver & Kempe, 1947; Demerec, 1948; Yegian & Vanderlinde, 1948; Newcombe & Hawirko, 1949; Meads & Haslam, 1949; English & McCoy, 1951; Mitchison, 1951; Olitzki, 1952), and that strains highly resistant to this antibiotic may be obtained in a single step (Demerec, 1948). The supposition that the rapid development of resistance to metachloridine in *P. gallinaceum* was due to the frequent occurrence, amongst the normal population, of variants possessing a high degree of resistance, was not supported experimentally. When survivors of a 50,000,000 inoculum, treated twice daily with doses of 0·8 mg. of the drug, were allowed to multiply in the absence of the drug, the population which resulted showed no increase in resistance to the drug as compared with the normal, untreated parasites. The criticism can be made that the initial inoculum was too small to include resistant forms, but this does not apply to the two strains, C and E, studied later, in which the initial inocula were large (0·5 or 0·3 ml. of heavily infected blood). These strains, maintained in birds treated with 0·025 mg. of metachloridine twice daily, were tested for resistance after varying periods of exposure to the drug. Resistance to this dosage developed rapidly, but parasites which grew well in birds so treated were not, at first, resistant to higher doses though they became so gradually.

The method by which a gradual increase in resistance to metachloridine developed in strains exposed over a period of time to 0·025 mg. doses of the drug is difficult to explain. Examination of blood films from infected birds undergoing treatment with such doses indicated that these were sufficiently large to affect a proportion of the parasites exposed to them; but resistance continued to develop after affected parasites could no longer be detected. However, phenomena of a somewhat similar kind have been described by workers in other fields of microbiology. Thus Silver & Kempe (1947) found that when a strain of *Escherichia coli* or *Aerobacter aerogenes* was cultivated on the chorioallantoic membrane of hen's eggs in the presence of subinhibitory doses of streptomycin, resistance to the drug increased; one strain, normally inhibited by 2 μg., was grown for 3 weeks in the presence of 1 μg., with the result that its resistance increased to over 10,000 μg.; another strain, normally inhibited by 8 μg., when grown in the presence of 5 μg. for 3 weeks, increased in resistance to 200 μg. Linz & Lane (1949) describe similar results with cultures of *Pseudomonas*

aeruginosa R exposed to streptomycin in concentrations greatly inferior to the bacteriostatic level. English & McCoy (1951), however, were unable to produce resistance to streptomycin in a strain of *Micrococcus pyogenes* var. *aureus* when the selective action of the drug was excluded; thus no increase in resistance was detected after eleven transfers through medium containing 0·01 unit of streptomycin. Gibson & Gibson (1951) found that when a strain of *Bacterium coli* was cultivated in a low concentration of dihydrostreptomycin (2·5 μg./ml.) an increase in resistance to approximately 10 times the concentration to which the organisms had been exposed, occurred; highly resistant cells also appeared, but their growth was not favoured by selection in the low concentration of antibiotic to which they were exposed though they became numerous if the concentration was increased. The experimental evidence for the development of a high grade of resistance to streptomycin in organisms cultivated in the presence of subinhibitory concentrations of the antibiotic, therefore, is conflicting, but the conditions of the experiments are not wholly comparable and the types of organism used differed.

It is not possible to follow the development of resistance to a drug in organisms maintained *in vivo* with the same precision as in those maintained *in vitro*. In studying the development of resistance to a drug in a strain of organism maintained by passage through living animals, such factors as variability in drug absorption amongst hosts, with resulting variability in the concentration of the drug to which the organism is exposed in the blood stream, and the effect of the natural immune reactions of the host upon the organism exposed to the drug, are beyond the control of the experimenter. However, the gradual development of resistance to doses of metachloridine greater than that to which the strain of *P. gallinaceum* was exposed, was clearly demonstrated.

The rate at which resistance to metachloridine developed in the strains passaged through chicks treated with 0·025 mg. doses of the drug was much slower than that of the strain which was passaged through birds receiving gradually increasing doses of the drug; in addition the final degree of resistance attained was not so high. It is, therefore, probable that the gradually increasing concentrations of the drug have a selective action upon the parasites, although no visible changes in the parasites were detected in the blood films. In the original strain which was passed through birds receiving gradually increasing doses of the drug, the intensity of infection produced in birds receiving 3·2 mg. continued to increase over a period of several months, although in the first tests made after the strain was exposed to this dose the parasites in birds receiving this dose appeared quite normal (Table 2). If the effect of the drug were to eliminate less resistant variants, it might be expected to exert its selective action in the first few passages after each increase in drug-concentration; the actual observations suggested a gradual increase in growth rate over a long period rather than a series of eliminations of less resistant individuals. It has been shown that in strains exposed to 0·025 mg. doses of the drug the population becomes resistant to a higher concentration of the drug than that to which it has been exposed, but that at higher concentrations growth is slow. If a strain so maintained were exposed to an increased concentration of the drug, the parasites would already be capable of growth at the new level, but individuals with a higher growth rate than the majority would be expected, gradually, to supersede the more slowly growing individuals. Under such conditions an increase in resistance would result from the selection of the most rapidly multiplying individuals.

It is of interest also that, as the growth rate of the original strain increased in birds receiving 3·2 mg. doses of the drug, its growth also increased, to some extent, in birds receiving low doses of sulphadiazine, sulphathiazole and sulphapyridine. The method by which this slight resistance to these drugs develops must differ from that by which resistance to these sulphonamides is produced by sulphadiazine itself, as in the metachloridine-resistant strain no cross-resistance to proguanil occurred.

The evidence obtained from cross-resistance tests indicated that the mode of action of metachloridine differs from that of proguanil, the sulphonamides, pamaquin, mepacrine or quinine. The rapidity with which resistance to metachloridine can develop in *P. gallinaceum* shows that the metabolic process which it inhibits can be side-tracked rapidly and efficiently.

6. SUMMARY

1. An increase in resistance to metachloridine of more than 100-fold was obtained within a few weeks in a strain of *Plasmodium gallinaceum* treated with gradually increasing doses of the drug and maintained in young chicks by blood-inoculation at intervals of 2–3 days.

2. There was no evidence that the rapid development of resistance arose by the selection of highly resistant individuals present in the normal population.

3. Two strains of *P. gallinaceum* passaged through chicks treated with 0·025 mg. doses of the drug gradually became resistant to greater concentrations than that to which they had been exposed, though their growth rate decreased when

they were inoculated into birds receiving higher doses of the drug.

4. In both strains maintained in birds treated with 0·025 mg. doses of the drug, resistance reached a maximum beyond which it did not increase.

5. Cross-resistance tests failed to show any relationship in mode of action between metachloridine and pamaquin, mepacrine, quinine or chloroquine. A strain of *P. gallinaceum*, highly resistant to metachloridine, showed slight resistance to sulphadiazine, sulphapyridine and sulphathiazole, but none to sulphanilamide or proguanil.

We are indebted to the Cyanamid Products Ltd., London, for the gift of the Folvite used in these experiments.

REFERENCES

ADAMS, A. R. D. & SEATON, D. R. (1949). Resistance to paludrine developed by a strain of *Plasmodium falciparum*. *Trans. R. Soc. Trop. Med. Hyg.* **42**, 314.

ALEXANDER, H. F. & LEIDY, G. (1947). Mode of action of streptomycin on type b *H. influenzae*. *J. Exp. Med.* **85**, 329.

BEERSTECHER, E. & SHIVE, W. (1947). Prevention of phenylalanine synthesis by tyrosine. *J. Biol. Chem.* **167**, 527.

BISHOP, A. (1951). Drug-resistance in Malaria. *Brit. Med. Bull.* **8**, 47.

BISHOP, A. & BIRKETT, B. (1947). Acquired resistance to paludrine in *Plasmodium gallinaceum*. Acquired resistance and persistence after passage through the mosquito. *Nature, Lond.*, **159**, 884.

BISHOP, A. & BIRKETT, B (1948). Drug-resistance in *Plasmodium gallinaceum*, and the persistence of paludrine-resistance after mosquito transmission. *Parasitology*, **39**, 126.

BISHOP, A. & MCCONNACHIE, E. W. (1948). Resistance to sulphadiazine and 'paludrine' in the malaria parasite of the fowl (*P. gallinaceum*). *Nature, Lond.*, **162**, 541.

BISHOP, A. & MCCONNACHIE, E. W. (1950*a*). Sulphadiazine-resistance in *Plasmodium gallinaceum* and its relation to other antimalarial compounds. *Parasitology*, **40**, 163.

BISHOP, A. & MCCONNACHIE, E. W. (1950*b*). Cross-resistance between sulphanilamide and paludrine (proguanil) in a strain of *Plasmodium gallinaceum* resistant to sulphanilamide. *Parasitology*, **40**, 175.

BISHOP, A. & MCCONNACHIE, E. W. (1952*a*). Failure to produce resistance to chloroquine in *Plasmodium gallinaceum* in chicks. *Parasitology*, **42**, 52.

BISHOP, A. & MCCONNACHIE, E. W. (1952*b*). Pamaquin-resistance in a strain of *Plasmodium gallinaceum* and its relationship to other antimalarial drugs. *Parasitology*, **42**, 57.

BRACKETT, S. & WALETZKY, E. (1946). The antimalarial activity of metachloridine (2-metanilamido-5-chloropyrimidine) and other metanilamide derivatives in test infections with *Plasmodium gallinaceum*. *J. Parasit.* **32**, 325.

COOPER, W. C., COATNEY, G. R. & IMBODEN, C. A. (1950). Studies in human malaria. XXIII. Acquired resistance to chlorguanide in the Chesson strain of *Plasmodium vivax*. *J. Nat. Malaria Soc.* **9**, 59.

DEMEREC, M. (1948). Origin of bacterial resistance to antibiotics. *J. Bact.* **56**, 63.

EDESON, J. F. B. & FIELD, J. W. (1950). Proguanil-resistant falciparum malaria in Malaya. *Brit. Med. J.* **1**, 147.

ENGLISH, A. R. & MCCOY, E. (1951). A study of streptomycin resistance in *Micrococcus pyogenes* var. *aureus*. *J. Bact.* **61**, 51.

FIELD, J. W. & EDESON, J. F. B. (1949). Paludrine-resistant falciparum malaria. *Trans. R. Soc. Trop. Med. Hyg.* **43**, 233.

FULTON, J. D. & YORKE, W. (1941). Studies in chemotherapy. XXIX. The development of plasmoquine-resistance in *Plasmodium knowlesi*. *Ann. Trop. Med. Parasit.* **35**, 233.

FULTON, J. D. & YORKE, W. (1943). Studies in Chemotherapy. Further observations on plasmoquine-resistance in *Plasmodium knowlesi*. *Ann. Trop. Med. Parasit.* **37**, 41.

GIBSON, M. I. & GIBSON, F. (1951). Development of resistance to dihydrostreptomycin by *Bacterium coli*. *Nature, Lond.*, **167**, 113.

VAN GOOR, W. T., LODENS, J. G. & GOMEZ, J. A. (1950). Clinical prophylaxis with proguanil and nivaquine in a community in Java. *Docum. neerl. indones. Morb. trop.* **2**, 341.

GREENBERG, J. (1949*a*). Inhibition of the antimalarial activity of chlorguanide by pteroylglutamic acid. *Proc. Soc. Exp. Biol., N.Y.*, **71**, 306.

GREENBERG, J. (1949*b*). The potentiation of the antimalarial activity of chlorguanide by *p*-aminobenzoic acid competitors. *J. Pharmacol.* **97**, 238.

GREENBERG, J., BOYD, B. L. & JOSEPHSON, E. S. (1948). Synergistic effect of chlorguanide and sulfadiazine against *Plasmodium gallinaceum* in the chick. *J. Pharmacol.* **94**, 60.

HAWKING, F. & PERRY, W. L. M. (1948). Resistance to proguanil (paludrine) in a mammalian malaria parasite (*Plasmodium cynomolgi*). *Lancet*, ii, 850.

HUGHES, C. O. & BRACKETT, S. (1946). The prevention of sporozoite-induced infections of *Plasmodium cathemerium* in the canary by metachloridine. *J. Parasit.* **32**, 340.

KLEIN, R. & KIMELMANN, L. J. (1946). The role of spontaneous variants in the acquisition of streptomycin resistance by the Shigellae. *J. Bact.* **52**, 471.

KNOPPERS, A. T. (1947). Acquired resistance (twofold) to quinine in *Plasmodium gallinaceum*. *Nature, Lond.*, **160**, 606.

KNOPPERS, A. T. (1949). Twofold quinine resistance of *Plasmodium gallinaceum*, induced by regular admini-

stration of the drug. *Docum. neerl. indones. Morb. trop.* **1**, 55.

LINZ, R. & LANE, L. (1949). Au sujet de la résistance bactérienne à la streptomycine après action de concentrations minimes de streptomycine. *C.R. Soc. Biol., Paris*, **143**, 726.

LOURIE, E. M. & SEATON, D. R. (1949). Resistance to paludrine developed by a strain of *Plasmodium vivax*. *Trans. R. Soc. Trop. Med. Hyg.* **42**, 315.

MEADS, M. & HASLAM, N. M. (1949). Quantitative studies on the origin and characteristics of streptomycin-fast variants of *Klebsiella pneumoniae*. *J. Immunol.* **63**, 1.

MITCHISON, J. A. (1951). The segregation of streptomycin-resistant variants of *Mycobacterium tuberculosis* into groups with characteristic levels of resistance. *J. Gen. Microbiol.* **5**, 596.

NEWCOMBE, H. B. & HAWIRKO, R. (1949). Spontaneous mutation to streptomycin resistance and dependence in *Escherichia coli*. *J. Bact.* **57**, 565.

OLITZKI, A. L. (1952). The determination of the most probable numbers of streptomycin-fast cells in Brucella cultures and their variability in growing and ageing cultures. *J. Gen. Microbiol.* **6**, 166.

ROLLO, I. M. (1951). A 2:4-diamino pyrimidine in the treatment of proguanil-resistant laboratory malarial strains. *Nature, Lond.*, **168**, 333.

SCHMIDT, L. H., GENTHER, C. S., FRADKIN, R. & SQUIRES, W. (1949). Development of resistance to chlorguanide (paludrine) during treatment of infections with *Plasmodium cynomolgi*. *J. Pharmacol.* **95**, 382.

SEATON, D. R. (1951). Failure to induce chlorquine-resistance in *Plasmodium gallinaceum*. *Ann. Trop. Med. Parasit.* **45**, 99.

SEATON, D. R. & ADAMS, A. R. D. (1949). Acquired resistance to proguanil in *Plasmodium gallinaceum*. *Lancet*, ii, 323.

SEATON, D. R. & LOURIE, E. M. (1949). Acquired resistance to proguanil (paludrine) in *Plasmodium vivax*. *Lancet*, i, 394.

SILVER, H. K. & KEMPE, C. H. (1947). Resistance to streptomycin. A study of the mechanisms of its development. *J. Immunol.* **57**, 263.

THOMPSON, P. E. (1948). On the ability of *Plasmodium lophurae* to acquire resistance to chlorguanide, camoquin and chloroquine. *J. Infect. Dis.* **83**, 250.

WILLIAMSON, J., BERTRAM, D. S. & LOURIE, E. M. (1947). Acquired resistance to paludrine in *Plasmodium gallinaceum*. Effects of paludrine and other antimalarials. *Nature, Lond.*, **159**, 885.

WILLIAMSON, J. & LOURIE, E. M. (1947). Acquired paludrine-resistance in *Plasmodium gallinaceum*. I. Development of resistance to paludrine and failure to develop resistance to certain other antimalarials. *Ann. Trop. Med. Parasit.* **41**, 278.

YEGIAN, D. & VANDERLINDE, R. J. (1948). A quantitative analysis of the resistance of mycobacteria to streptomycin. *J. Bact.* **56**, 177.

(*MS. received for publication* 28. v. 1952.—Ed.)

POLYHEDRAL VIRUS DISEASES AFFECTING THE LARVAE OF THE PRIVET HAWK MOTH (*SPHINX LIGUSTRI*)

By KENNETH M. SMITH, R. W. G. WYCKOFF and N. XEROS, *Agricultural Research Council, Plant Virus Research Unit, Molteno Institute, University of Cambridge, and Laboratory of Physical Biology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, Federal Security Agency, Bethesda, Maryland.*

(With Plates VI—VIII)

INTRODUCTION

During June 1951 a quantity of eggs of the privet hawk moth were obtained in Cambridge. On emergence from the egg it was noticed that a number of the young larvae had died, some immediately after emerging, others after a period of a few days. In some cases the young larvae died before they had got completely clear of the egg shell. Examination of these larvae showed that all had died of a polyhedral virus disease.

DESCRIPTION OF THE DISEASE

The insect is most susceptible to this disease during its early larval stages and until it is half-grown. During this period the disease is highly infectious and rapidly fatal, its incubation period averaging about 10 days. It follows a course fairly typical of this kind of infection: the larva first ceases to feed and remains motionless on a stem of the food plant or at the bottom of the cage. After this, the disease develops rapidly and the larva becomes limp and flaccid. Its skin is apparently very thin and easily ruptures, liberating large quantities of fluid containing vast numbers of polyhedral crystals. Very young larvae, about 10 mm. in length, when naturally infected, sometimes showed a dark patch in the abdomen. After the larva is half-grown, however, the situation as regards infection is rather different. Such older larvae appear to be much less susceptible but can still be infected. When this happens they enter on what can be described as a static condition which may last for as long as 4 or 5 weeks. All growth ceases, although a little feeding may take place; they seem to pass through the normal number of instars and appear similar to mature larvae, but usually are less than a half the normal size (Pl. VI, fig. 1). If a blood smear is made from one of these caterpillars, a few polyhedra can usually be found but nothing like the number characteristic of earlier infections. The larva eventually dies but here again conditions are different. The skin remains tough and the caterpillar has a desiccated appearance instead of the moist flaccid condition found in diseased younger caterpillars; as a rule only a few polyhedra are present. When a larva has become infected late in its development, it frequently pupates, forming a normal-appearing chrysalis. This soon dies, however, and examination shows the pupa to be practically empty, containing only numbers of polyhedral bodies.

Most of the polyhedra produced during this disease have been rather large and roughly rectangular in outline. Starting in the nuclei of the fat or blood cells, only one or two at first are visible in a cell (Pl. VI, fig. 2); but as the disease progresses, these cells become completely filled with polyhedra and eventually burst (Pl. VI, fig. 3).

Several more seemingly spontaneous cases of the disease have occurred since the virus was first observed in the young larvae in June 1951. Indeed, it appears to be of widespread occurrence in this species, especially in caterpillars raised in captivity by dealers. It seems clear that this virus can be passed from parent to offspring since the larvae died of the disease in some cases before complete emergence from the egg had taken place.

When making blood smears from infected caterpillars and staining with methylene blue, it was noticed that some of the polyhedra readily took the stain though others, behaving in the usual fashion, remained unstained (Pl. VII, fig. 4). In previous work we have found that the polyhedra from different kinds of diseased caterpillars showed this difference in staining, and with it a different reaction to treatment with weak alkali. This naturally suggests that in the present case we may be concerned with a caterpillar simultaneously infected with two different polyhedral diseases. To those familiar with the virus diseases of plants, the occurrence of two or more viruses acting together in one plant is commonplace. This is, however, the first evidence we had of such a dual infection of insects with polyhedral diseases.

Of the polyhedral diseases previously studied those giving non-staining polyhedra are represented

by the ones that attack the silkworm, *Bombyx mori*, and the caterpillar of the scarlet tiger moth, *Panaxia dominula*. When treated with dilute sodium carbonate these disintegrate rather completely, leaving behind a membrane, a fine grained uniform macromolecular residue and frequently many rod-shaped particles which are generally considered to be the units of viral activity. The other type of polyhedron, which readily takes up methylene blue, was first found in diseased larvae of the garden tiger moth, *Arctia caja*. It dissolves much less completely in sodium carbonate, leaving both in this case and in that of *Rhypara purpurata* a coarsely granular residue, a polyhedral shell dotted with holes and often many spherical particles of approximately uniform size which could be units of the viral activity (Smith & Wyckoff, 1951).

The non-staining and staining polyhedra here found together in diseased larvae of the privet hawk moth do not behave in a similar manner in their reactions to sodium carbonate. Pl. VII, fig. 5, shows a non-staining polyhedron which has been partially disintegrated through treatment with 2% sodium carbonate for 30 sec.; its enveloping membrane and the fine texture of the extruded polyhedral contents are clear. Neither in this nor in other photographs of these alkalinized polyhedra are particles visible which can properly be identified with the virus. A typical photograph of the blue-staining polyhedra is given in Pl. VII, fig. 6. They have been exposed to ½% sodium carbonate for 45 sec. The very different texture of their contents is apparent. They are in coarse lumps with holes similar in size to those seen in *Arctia caja*. An enveloping membrane apparentiy is missing. The only significant difference from the other staining polyhedra lies in the fact that the residual shell here has collapsed, instead of retaining more or less the shape of the original polyhedron. This collapse apparently is characteristic rather than accidental, for of the many polyhedral residues from *Sphinx ligustri* appearing in our electron micrographs, all are, without exception, flattened.

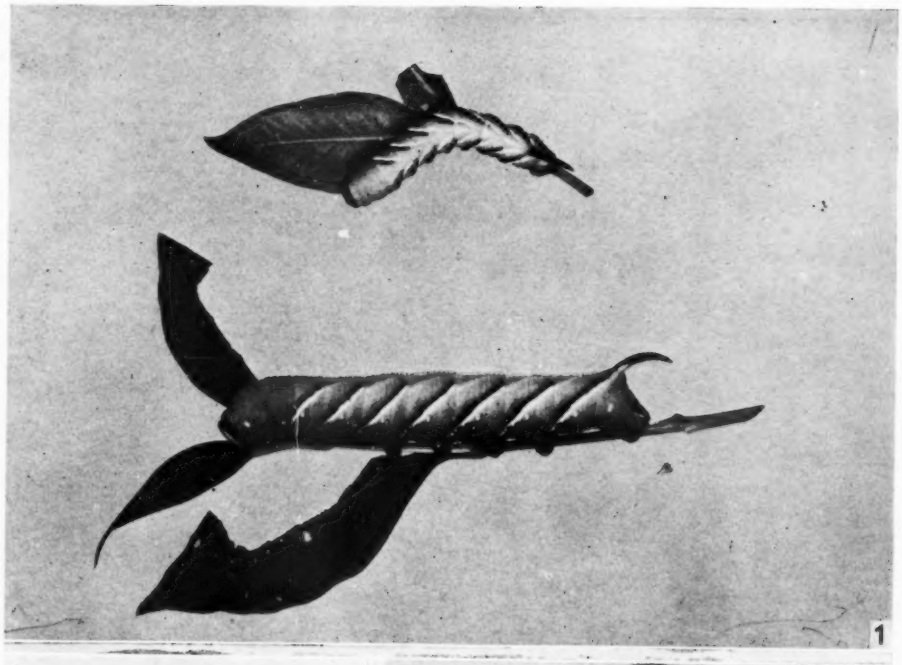
A survey has also been made of the development of the *S. ligustri* polyhedra through the electron microscopy of thin sections of infected larvae. For this purpose the larvae were fixed in either Carnoy's fluid or neutral formalin, the latter giving the better preparation. Small pieces of the fixed larvae were dehydrated, embedded in methacrylate and thinly sectioned, using a rotary microtome adapted for expansion cutting. These sections, mounted on formvar-covered grids, were treated with amyl acetate to remove the plastic and shadowed lightly with palladium before being examined in a type EMU-2 electron microscope manufactured by R.C.A. The electron micrographs thus obtained show some of the steps whereby the polyhedra develop. The most interesting feature seen with this disease is probably the 'honeycomb' often observed in the nuclei of infected cells. An example is shown in Pl. VIII, fig. 7. Here one sees very clearly the pockets of the honeycomb with their opaque inclusions, which this and other photographs suggest are polyhedra in an early stage of formation. Pl. VIII, fig. 8, shows a nucleus with two seemingly empty cells and a number of well-formed polyhedra not included in pockets. It may be that the cells appear empty only because their inclusions are at a level other than that appearing in the section photographed. Though the fully formed polyhedra in these two photographs are not enclosed in pockets, such polyhedra sometimes are pocketed. Several of these appear in Pl. VIII, fig. 9. Similar 'honeycombs' have been seen in sections through other infected lepidopterous larvae, notably those of *Telea polyphemus*. Most of the sectioned polyhedra, when thin enough to be partly transparent, have been devoid of fine detail but some have contained granules which could conceivably be particles of virus (Pl. VIII, fig. 10).

DISCUSSION

The present observations are specially important for their bearing on the relation between the polyhedral crystal and the virus responsible for the disease producing it. Whatever this relation may be, the crystal must be a product of the response of the cell to the infecting virus since it is not the virus itself. What is needed is a knowledge of how far the host and how far the virus is dominant in fixing the character of the polyhedral body. The study of cross- and multiple-infection is the key to this question.

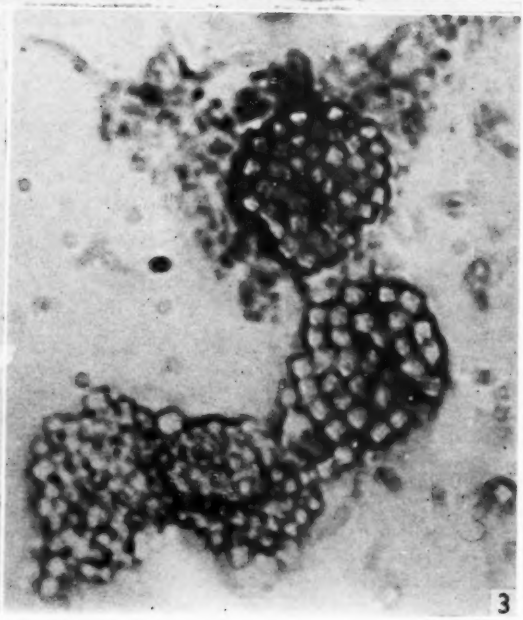
It has generally been considered that the polyhedral diseases were so strongly species-specific that cross-infection could not be brought about, even between very closely related species. Some recent work by Smith & Xeros (1952*a*), however, casts strong doubt on the validity of such a generalization. They found several examples of cross-infection between distantly related species; thus the privet hawk caterpillar has been infected with the virus from the larvae of *Panaxia dominula*, the scarlet tiger moth; *Arctia villica*, the cream spot tiger moth, *A. grossulariata*, the gooseberry or currant moth; *Cycnia mendica*, the muslin moth; the two silk moths, *Philosamia ricini* and *Telea polyphemus*; and with a new virus from the larva of the painted lady butterfly, *Pyrameis cardui*.

The fact that in the case of the present, seemingly dual, infection of *Sphinx ligustri*, two very different kinds of polyhedra are formed at the same time, suggests that the virus may be especially effective in determining the character of the polyhedra to which its disease gives rise. This, in turn, points to the desirability of experiments in which an attempt is made to infect a caterpillar simultaneously with two diseases characterized by the two kinds of polyhedra

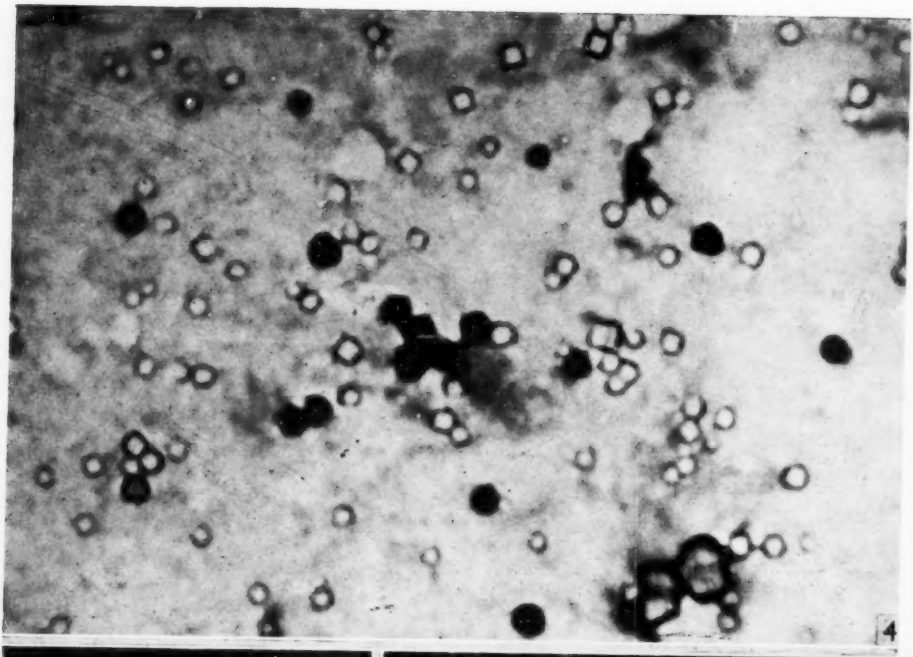


1

2

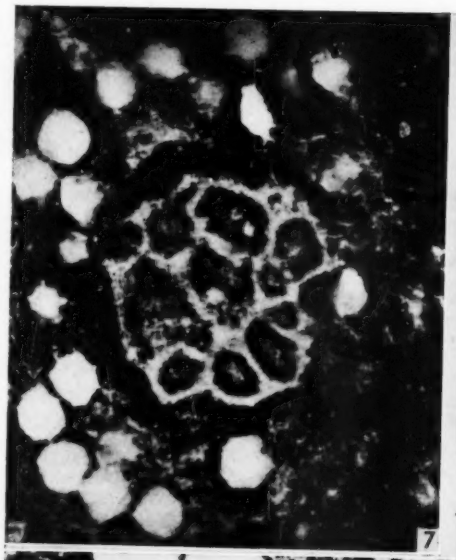


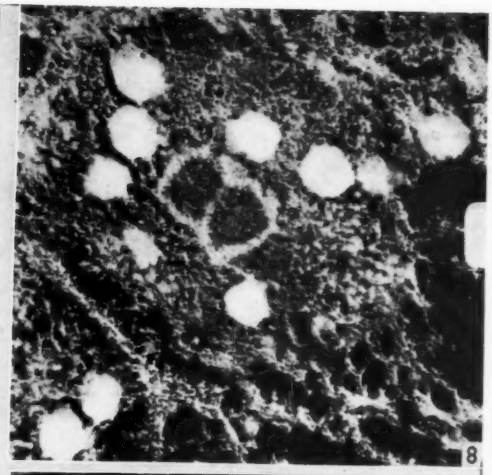
3

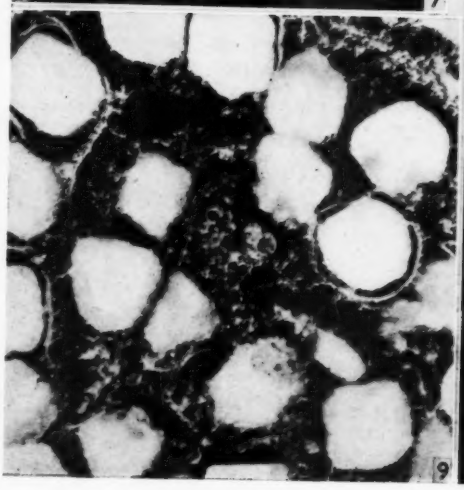

4

5

6


7


8


9

10

described above. An example of such a proposed experiment would be the infection of the privet hawk caterpillar with the viruses of *Panaxia dominula* (which has membrane-covered, fine-grained polyhedra) and of *Arctia villica* (that has membrane-less, coarse-grained polyhedra). Such experiments are under way. As throwing further light on this question it is relevant here to mention that we have succeeded in infecting silkworms (*Bombyx mori*) with the viruses from *Rhypara purpurata* and the painted lady (*Pyrameis cardui*). The polyhedra formed in the silkworm with the virus from *Rhypara purpurata* are very similar to, but not quite identical with, the blue-staining, coarsely granular type of polyhedra characteristic of the *purpurata* virus (Smith & Xeros, 1952*b*).

REFERENCES

SMITH, KENNETH M. & WYCKOFF, R. W. G. (1951). Electron microscopy of insect viruses. *Research, Lond.*, **4**, 148–155.

SMITH, KENNETH, M. & XEROS, N. (1952*a*). Transmission of polyhedral viruses between different insect species. *Nature, Lond.*, **170**, 492.

SMITH, KENNETH, M. & XEROS, N. (1952*b*). Cross- and multiple-infection experiments with polyhedral viruses. *Parasitology* (in the Press).

EXPLANATION OF PLATES VI–VIII

PLATE VI

Fig. 1. Two larvae of *Sphinx ligustri*, the privet hawk moth; both larvae are of the same age, that above is in the static condition of virus infection. Slightly enlarged.

Fig. 2. Blood cell from an infected larva at an early stage of polyhedral infection. × 850.

Fig. 3. Blood cells in a later stage of infection, showing the polyhedral bodies bursting out of the cells. × 850.

PLATE VII

Fig. 4. Blood smear from a larva infected with a dual disease; note the staining and non-staining types of polyhedra. × 850.

Fig. 5. A single polyhedral crystal of the non-staining type, photographed on the electron microscope; note the encircling membrane and fine structure of the extruded contents. × 17,500.

Fig. 6. Three polyhedra of the staining type; note absence of membrane and coarse granular structure. × 21,000.

PLATE VIII

Fig. 7. 'Honeycomb' structure in the cell; note a polyhedral body apparently emerging from one of the pockets. × 9,000.

Fig. 8. Two apparently empty pockets of the honeycomb structure with fully formed polyhedra nearby. × 10,000.

Fig. 9. Section through infected cell, showing 'pocketing' of some of the polyhedra. × 10,000.

Fig. 10. Very thin sections of polyhedra showing small particles which might be virus particles. × 10,000.

INDEX OF AUTHORS

ARTHUR, D. R. *Ixodes frontalis* Panzer, 1795. A review of the species. (With 8 Figures in the Text), 151

ARTHUR, D. R. *Ixodes passericola* Schulze. A description of the hitherto unknown male and larva, with a re-description of the female and nymph. (With 17 Figures in the Text), 155

ARTHUR, D. R. The morphology of the British Prostriata with particular reference to *Ixodes hexagonus* Leach. II. (With 40 Figures in the Text), 161

ARTHUR, D. R. The capitulum and feeding mechanism of *Ixodes hexagonus* Leach. II. (With 7 Figures in the Text), 187

BISHOP, ANN and MCCONNACHIE, ELSPETH W. Failure to produce resistance to chloroquine in *Plasmodium gallinaceum* in chicks, 52

BISHOP, ANN and MCCONNACHIE, ELSPETH W. Pamaquin resistance in a strain of *Plasmodium gallinaceum* and its relationship to other antimalarial drugs, 57

BISHOP, ANN and MCCONNACHIE, ELSPETH W. The development of resistance to metachloridine in *Plasmodium gallinaceum* in chicks, 277

BROZ, O. and PRIVORA, M. Two skin parasites of *Rana temporaria*: *Dermocystidium ranae* Guyénot & Naville and *Dermosporidium granulosum* n.sp. (With Plates II and III and 2 Figures in the Text), 65

CABLE, R. M. On the systematic position of the genus *Deropristis*, of *Dihemistephanus sturionis* Little, 1930, and of a new digenetic trematode from a sturgeon. (With 8 Figures in the Text), 85

CROFTON, H. D. The ecology of immature phases of trichostrongyle nematodes. IV. Larval populations on lowland pastures. (With 5 Figures in the Text), 77

DOBELL, the late CLIFFORD. Researches on the intestinal Protozoa of monkeys and man. XII. Bacterial factors influencing the life history of *Entamoeba histolytica* in cultures. (With 1 Figure in the Text), 16

DOUGHERTY, ELLSWORTH C. Problems of nomenclature for the growth of organisms of one species with and without associated organisms of other species, 259

DSCHUNKOWSKY, E. Remarks on theilerioses and *Theileria*, 70

DUTT, S. C. and SRIVASTAVA, H. D. On the morphology and life history of a new mammalian blood-fluke—*Ornithobilharzia dattai* n.sp. (Preliminary report.) (With 11 Figures in the Text), 144

GIBBS, ALFRED J. A sporozoan blood parasite of *Archiulus moreleti* (Diplopoda). (With 13 Figures in the Text), 74

GUGGISBERG, C. A. W. *See* HEISCH, R. B.

HEISCH, R. B. and GUGGISBERG, C. A. W. On *Ornithodorus graingeri* n.sp., a tick from caves in Kenya. (With 19 Figures in the Text), 192

HOARE, CECIL A. *See* MACKINNON, DORIS L.

HOARE, CECIL A. The food habits of *Entamoeba histolytica* in its commensal phase. (With 1 Figure in the Text), 43

JOBLING, B. Description of two new species of *Ascodipteron* from Africa and one species of *Nycteribosca* from Madagascar (Diptera, Streblidae). (With 4 Figures in the Text), 126

KUPER, S. W. A. and PELC, S. R. Radioactive labelling experiments with *Plasmodium*. (With Plate V), 269

LAWRENCE, R. F. Two new mite parasites of Natal bats (Listrophoridae, Sarcoptiformes). (With 4 Figures in the Text), 136

LUBINSKY, GEORGE. The occurrence in Pakistan of a human *Entamoeba* of the *polecki* type. (With 9 Figures in the Text), 48

MACKINNON, DORIS L. and HOARE, CECIL A. *Clifford Dobell* (1886–1949). In Memoriam, 1

MCCONNACHIE, ELSPETH W. *See* BISHOP, A.

MCCONNACHIE, ELSPETH W. The action of 2:4-diamino-6:7-di*iso*propylpteridine on normal, proguanil- and sulphadiazine-resistant strains of *Plasmodium gallinaceum*, 272

NEAL, R. A. Experimental production of pure mixed strains of *Entamoeba histolytica*, 40

PELC, S. R. *See* KUPER, S. W. A.

PRIVORA, M. *See* BROZ, O.

RADFORD, CHARLES D. The mites (Acarina: Analgesidae) living on or in the feathers of birds, 199

RADFORD, CHARLES D. Five new species of larval mites (Acarina: Trombiculinae). (With 10 Figures in the Text), 231

RADFORD, CHARLES D. Four new species of parasitic mites (Acarina). (With 11 Figures in the Text), 239

REES, GWENDOLEN. The structure of the adult and larval stages of *Plagiorchis* (*Multiglandularis*) *megalorchis* n.nom. from the turkey and an experimental demonstration of the life history. (With 46 Figures in the Text), 92

REES, GWENDOLEN. Spiral torsion in *Taenia taeniaeformis* (Batsch, 1786) caused by the knotting together of two worms. (With 24 Figures in the Text), 114

SARWAR, M. M. On the synonymy of *Marshallagia orientalis* (Bhalerao, 1932) with *Marshallagia marshalli* and a record of its occurrence from the mountainous region of the Punjab, 125

SMITH, KENNETH M., WYCKOFF, R. W. G. and XEROS, N. Polyhedral virus diseases affecting the larvae of the privet hawk moth (*Sphinx ligustri*). (With Plates VI–VIII), 287

SPRENT, J. F. A. On the life history of *Ascaris devosi* and its development in the white mouse and the domestic ferret. (With Plate IV and 28 Figures in the Text), 244

SRIVASTAVA, H. D. *See* DUTT, S. C.

THOMAS, MAIR E. M. Observations upon the effects of mepacrine and other substances on *Giardia intestinalis*, 262

WYCKOFF, R. W. G. *See* SMITH, KENNETH M.

XEROS, N. *See* SMITH, KENNETH M.

INDEX OF SUBJECTS

Acarina: Analgesidae, the mites, living on or in the feathers of birds, 199
Acarina: four new species of parasitic mites, 239
Acarina: Trombiculinae, five new species of larval mites, 231
Archiulus morelati Diplopoda, a sporozoan blood parasite of, 74
Ascaris devosi, life history of, and its development in the white mouse and domestic ferret, 244
Ascodipteron, description of two new species of, from Africa and one species of *Nycteribosca* from Madagascar (Diptera, Streblidae), 126

Bacterial factors influencing the life history of *Entamoeba histolytica* in culture, 16
Birds, mites living on or in the feathers of, 199
British Prostriata, morphology of the, 161

Capitulum and feeding mechanism of *Ixodes hexagonus* Leach, 187
Chloroquine, failure to produce resistance to, in *Plasmodium gallinaceum* in chicks, 52

Dermocystidium ranae Guyénot & Naville and *Dermosporidium granulosum* n.sp., two skin parasites of *Rana temporaria*, 65
Deropristis, on the systematic position of the genus, of *Dihemistephanus sturionis* Little, 1930, and of a new digenetic trematode from a sturgeon, 85
Dobell, Clifford. In memoriam, 1

Ecology of immature phases of Trichostrongyle nematodes. IV. Larval populations on lowland pastures, 77
Entamoeba histolytica, experimental production of pure mixed strains of, 40
Entamoeba histolytica in cultures, bacterial factors influencing the life history of, 16
Entamoeba histolytica, in its commensal phase, the food habits of, 43
Entamoeba of the *polecki* type, occurrence in Pakistan of a human, 48

Feeding mechanism of *Ixodes hexagonus*, 187
Ferret, development of *Ascaris devosi* in the domestic, 244

Giardia intestinalis, the effects of mepacrine and other substances on, 262

Intestinal Protozoa of monkeys and man, 16
Ixodes frontalis Panzer, 1795. A Review of the species, 151
Ixodes hexagonus Leach. Capitulum and feeding mechanism of, 187
Ixodes hexagonus Leach. The morphology of the British Prostriata with particular reference to, 161
Ixodes passericola Schulze. A description of the hitherto unknown male and larva, with a re-description of the female and nymph, 155

Kenya, a tick from caves in, 192

Larval mites, five new species of, 231
Life history of a new mammalian blood fluke, *Ornithobilharzia dattai* n.sp., 144
Life history of *Ascaris devosi* and its development in the white mouse and domestic ferret, 244
Life history of *Plagiorchis* (*Multiglandularis*) *megalorchis* n.nom. from the turkey, 92
Listrophoridae, Sarcoptiformes, Natal Bats, two new mite parasites of, 136

Marshallagia orientalis (Bhalerao, 1932), on the synonymy of, with *Marshallagia marshalli* and a record of its occurrence from the mountainous regions of the Punjab, 125
Mepacrine and other substances, the effects of, on *Giardia intestinalis*, 262
Metachloridine, development of resistance to, in *Plasmodium gallinaceum* in chicks, 277
Mite parasites, two new, of Natal Bats, Listrophoridae, Sarcoptiformes, 136
Mites (Acarina: Analgesidae) living on or in the feathers of birds, 199
Mites, five new species of larval, 231
Mites, four new species of parasitic, 239
Morphology and life history of a new mammalian blood-fluke, *Ornithobilharzia dattai* n.sp., 144
Morphology of the British Prostriata with particular reference to *Ixodes hexagonus* Leach, 161
Mouse, development of *Ascaris devosi* in the white, 244

Nomenclature, problems of, for the growth of organisms of one species with and without associated organisms of other species, 259

Ornithobilharzia dattai n.sp., on the morphology and life history of a new mammalian blood-fluke, 144
Ornithodoros graingeri n.sp., a tick from caves in Kenya, 192

Pamaquin resistance in a strain of *Plasmodium gallinaceum* and its relationship to other antimalarial drugs, 57
Plagiorchis (*Multiglandularis*) *megalorchis* n.nom., the structure of the adult and larval stages of, from the turkey and an experimental demonstration of the life history, 92
Plasmodium gallinaceum in chicks, development of resistance to metachloridine in, 277
Plasmodium gallinaceum in chicks, failure to produce resistance to chloroquine in, 52

Plasmodium gallinaceum, pamaquin resistance in a strain of, 57
Plasmodium gallinaceum, the action of 2:4-diamino-6:7-di*iso*propylpteridine on normal, proguanil- and sulphadiazine-resistant strains of, 272
Plasmodium, radioactive labelling experiments with, 269
Polyhedral virus diseases affecting the larvae of the privet hawk moth (*Sphinx ligustri*), 287
Privet hawk moth, polyhedral virus diseases affecting the larvae of the, 287
Prostriata, morphology of the British, 161
Protozoa of monkeys and man, researches on the intestinal, 16

Radioactive labelling experiments with *Plasmodium*, 269
Rana temporaria, two skin parasites of, *Dermocystidium ranae* Guyénot & Naville and *Dermosporidium granulosum* n.sp., 65

Sphinx ligustri, the privet hawk moth, polyhedral virus diseases affecting the larvae of, 287
Sporozoan blood parasite of *Archiulus morelati* (Diplopoda), 74
Synonymy of *Marshallagia orientalis* (Bhalerao, 1932) with *Marshallagia marshalli* and a record of its occurrence from the mountainous regions of the Punjab, 125
Systematic position of the genus *Deropristis*, of *Dihemistephanus sturionis* Little, 1930, and of a new digenetic trematode from a sturgeon, 85

Taenia taeniaeformis (Batsch, 1786), spiral torsion in, caused by the knotting together of two worms, 114
Theileria, remarks on theilerioses and, 70
Trematode, a new digenetic, from a sturgeon, 85
Trichostrongyle nematodes, ecology of immature phases of, IV. Larval populations on lowland pastures, 77
Trombiculinae, five new species of larval, 231

Virus diseases, polyhedral, affecting the larvae of the privet hawk moth, 287

Publications issued by

COMMONWEALTH INSTITUTE OF ENTOMOLOGY

41 Queen's Gate, London, S.W. 7

BULLETIN OF ENTOMOLOGICAL RESEARCH

Published quarterly and containing original articles on Economic Entomology. *Issued Post Free.*

Annual Subscription (payable in advance). Vol. 43 (1952) et sqq. 60*s.* 0*d.*

Prices of back parts and volumes on application.

REVIEW OF APPLIED ENTOMOLOGY

Consisting of abstracts or reviews of all current literature on Economic Entomology throughout the world. Published monthly in two series:

Series "A" dealing with insect and other Arthropod pests of cultivated plants, forest trees and stored products of animal and vegetable origin.

Series "B" dealing with insects, ticks, etc. conveying disease or otherwise injurious to man and animals. *Issued Post Free.*

	Series "A"	*Series "B"*
Annual Subscription (payable in advance)	40*s.* 0*d.*	20*s.* 0*d.*

Prices of back parts and volumes on application.

ZOOLOGICAL RECORD, part INSECTA

Published annually about October each year and containing as complete a record as possible of the literature of the previous year, chiefly from the systematic standpoint.

Annual Subscription (including postage) vol. 86 et sqq. 30*s.* 6*d.*

Prices of back volumes on application.

Parasitic Animals

GEOFFREY LAPAGE

An attempt to give, from the biological point of view, an outline of the general principles which govern the lives of parasitic animals, illustrated mainly by reference to those species which attack man or the domesticated animals, with a view to showing the nature and extent of the losses inflicted by parasites on human civilization. A new volume in the Cambridge Library of Modern Science. 21*s. net*

CAMBRIDGE UNIVERSITY PRESS

CONTENTS

(*All rights reserved*)

PAGE

ARTHUR, D. R. The morphology of the British Prostriata with particular reference to *Ixodes hexagonus* Leach. II. (With 40 Figures in the Text) . . . 161

ARTHUR, D. R. The capitulum and feeding mechanism of *Ixodes hexagonus* Leach. II. (With 7 Figures in the Text) 187

HEISCH, R. B. and GUGGISBERG, C. A. W. On *Ornithodorus graingeri* n.sp., a tick from caves in Kenya. (With 19 Figures in the Text) 192

RADFORD, CHARLES D. The mites (Acarina: Analgesidae) living on or in the feathers of birds 199

RADFORD, CHARLES D. Five new species of larval mites (Acarina: Trombiculinae). (With 10 Figures in the Text) 231

RADFORD, CHARLES D. Four new species of parasitic mites (Acarina). (With 11 Figures in the Text) 239

SPRENT, J. F. A. On the life history of *Ascaris devosi* and its development in the white mouse and the domestic ferret. (With Plate IV and 28 Figures in the Text) 244

DOUGHERTY, ELLSWORTH C. Problems of nomenclature for the growth of organisms of one species with and without associated organisms of other species . . 259

THOMAS, MAIR E. M. Observations upon the effects of mepacrine and other substances on *Giardia intestinalis* 262

KUPER, S. W. A. and PELC, S. R. Radioactive labelling experiments with *Plasmodium*. (With Plate V) 269

MCCONNACHIE, ELSPETH W. The action of 2:4-diamino-6:7-di*iso*propylpteridine on normal, proguanil- and sulphadiazine-resistant strains of *Plasmodium gallinaceum* 272

BISHOP, ANN and MCCONNACHIE, ELSPETH W. The development of resistance to metachloridine in *Plasmodium gallinaceum* in chicks 277

SMITH, KENNETH M., WYCKOFF, R. W. G. and XEROS, N. Polyhedral virus diseases affecting the larvae of the privet hawk moth (*Sphinx ligustri*). (With Plates VI–VIII) 287

Index of Authors 291

Index of Subjects 293

PARASITOLOGY is published three or four times a year. The numbers afterwards are issued in volumes each containing four numbers.

Papers for publication should be sent to Professor D. KEILIN, Molteno Institute, Downing Street, Cambridge. Other communications should be addressed to the University Press, Cambridge.

Papers forwarded to the Editors for publication are understood to be offered to *PARASITOLOGY* alone, unless the contrary is stated.

Contributors receive fifty copies of their papers free. Additional copies, not exceeding one hundred (except in special cases), may be had at cost price: these should be ordered when the final proof is returned.

The subscription price for each volume after volume 42, payable in advance, is £3. 15*s*. net per volume (post-free) single numbers 21*s*. net (double numbers 42*s*. net) postage extra. Subscriptions may be sent to any Bookseller, or to The Cambridge University Press, Bentley House, 200 Euston Road, N.W. 1.

Enquiries from the U.S.A. should be addressed to the American Branch of the Press at 32 East 57th Street, New York, 22.

Printed in Great Britain at the University Press, Cambridge
(Brooke Crutchley, University Printer)